University of Montreal

Expression and regulation of microsomal prostaglandin E synthase-1 in human osteoarthritic cartilage and chondrocytes

By

Xinfang Li

Department of Biomedical Sciences

Faculty of Medicine

This thesis was submitted to the Faculty of Graduate studies and Research in partial fulfilment of the requirments of the degree of Master in Biomedical Sciences.

August 2005

University of Montreal
Faculty of graduate studies

This thesis entitled

Expression and regulation of microsomal prostaglandin E synthase-1 in human osteoarthritic cartilage and chondrocytes

Presented by
Xinfang Li

Has been evaluated by a jury made up of the following people

President of jury : Dr. Muhammad Zafarullah

Director of research : Dr. Hassan Fahmi

Member of jury : Dr. Mohamed Benderdour

August 2005

Summary

Prostaglandin E_2 (PGE_2) is the major prostanoid synthesized in the joint and play an important role in inflammation and pathogenesis of arthritis. High concentrations of PGE_2 have been detected in serum and synovial fluids from arthritic patients. In the PGE_2 biosynthesis pathway, the synthesis of PGE_2 from arachidonic acid (AA) requires 2 enzymes acting sequentially. Cyclooxygenases catalyze the conversion of AA to the intermediate prostanoid PGH_2. Subsequently, PGES converts COX-derived PGH_2 into PGE_2.. At least three distinct PGES isoforms have been identified, which are called microsomal PGES-1 (mPGES-1), mPGES-2, and cytosolic PGES. Among them, mPGES-1 is induced by various inflammatory stimuli in some cells and tissues and exhibits preferential functional coupling with COX-2. Pro-inflammatory cytokines IL-1β and TNF-α have been shown to induce mPGES-1 expression in several tissues and cell types. However, little is known about the expression and regulation of mPGES-1 in cartilage.

In order to better understand the regulation of PGE_2 production in joint tissues, we analyzed mPGES-1 expression in nomal and OA cartilage. Furthemore, we explored the effects of different inflammatory agonists on the expression of mPGES-1 in OA chondrocytes and tested the effect of 15-PGJ_2 on IL-1β-induced mPGES-1 expression in OA chondrocytes. Our present study showed that levels of mPGES-1 mRNA and protein were markedly elevated in OA versus normal human cartilage. Treatment of chondrocyte with IL-1β induced the expression of mPGES –1 protein in a dose- and time-dependent manner. This appears to occur at the transcriptional level as IL-1β induced the expression of mPGES-1 mRNA and the activity of this gene promoter. Furthermore, TNF-α and IL-17 also up-regulated the expression of mPGES-1 protein and displayed a synergistic effect with IL-1β. The results obtained with 15-PGJ_2 and PGE_2 on mPGES protein expression of chondrocytes were also interesting. We showed that 15-PGJ_2 inhibits IL-1β-induced mPGES-1 protein expression, an effect that was reversed by exogenous PGE_2.

To conclude, our study shows that mPGES-1 expression is up-regulated in OA versus normal cartilage, proinflammatory cytokines increased mPGES-1 expression and PPARγ ligand 15d-PGJ_2 repressed IL-1β-induced mPGES-1 expression in chondrocytes. These data suggest that mPGES-1 may prove to be an interesting therapeutic target for controlling PGE_2.

Key words:

Osteoarthritis, Microsomal prostaglandin E synthase-1, Cartilage, Chondrocytes, PGE_2, PPARγ, and 15d-PGJ_2.

RÉSUMÉ

La prostaglandine E_2 est une prostanoïde majeure synthétisée dans l'articulation. Celle-ci joue un rôle important dans l'inflammation et dans la pathogenèse de l'arthrite. De hautes concentrations de PGE_2 ont été détectées dans le sérum et dans les liquides synoviaux des patients arthritiques. Dans la voie de la biosynthèse de la PGE_2, la synthèse de la PGE_2 à partir d'acide arachidonique (AA) nécessite l'action séquentielle de deux enzymes. La cyclooxygénase catalyse la conversion de l'AA en prostanoïde intermédiaire PGH_2. Ensuite, la PGE synthase (PGES) convertit la PGH_2 dérivée de la COX-2 en PGE_2. Pas moins de trois isoformes distincts de PGES ont été identifiés : PGES-1 microsomal (mPGES-1), mPGES-2 ainsi que PGES cytosolique. Parmi eux, mPGES-1 est induite par divers stimuli inflammatoires dans certaines cellules et certain tissus, et elle est préférentiellement couplée à COX-2.

Il a été démontré que les cytokines pro-inflammatoires IL-1β et TNF-α induisent l'expression de la mPGES-1 dans plusieurs tissus et types de cellules. Toutefois, très peu est connu au sujet de l'expression et de la régulation de la mPGES-1 dans le cartilage.

Dans le but de mieux comprendre la régulation de la production de la PGE_2 dans les tissus de l'articulation, nous avons analysé l'expression de la mPGES-1 dans le cartilage normal et celui ostéoarthritique (OA). De plus, nous avons exploré les effets que différents agonistes ont sur l'expression de la mPGES-1 dans les chondrocytes OA et nous avons testé l'effet de la 15-PGJ_2 sur la mPGES-1 induite par IL-1β dans les chondrocytes OA. La présente étude démontre que les niveaux d'ARNm et de protéines de mPGES-1 sont élevés dans le cartilage OA comparativement au cartilage normal. Le traitement des chondrocytes avec IL-1β induit l'expression de la protéine mPGES-1 de manière dose et temps dépendante. Ceci semble survenir à l'étape de transcription au cours de laquelle IL-1β induit l'expression l'ARNm de mPGES-1 et l'activité de ce promoteur de gène. De plus,

TNF-α et IL-17 augmente aussi l'expression de la protéine mPGES-1 et possède un effet synergique avec IL-1β. Les résultats obtenus avec 15-PGJ_2 et PGE_2 sur l'expression de la protéine mPGES des chondrocytes sont également intéressants. Nous avons démontré que 15-PGJ_2 inhibe l'expression de la protéine mPGES-1 induite par IL-1β, un effet qui a été contré par la PGE_2 exogène.

En conclusion, notre étude démontre que la mPGES-1 est exprimé de manière plus significative dans le cartilage OA que dans le cartilage normal. De plus, les cytokines pro-inflammatoires augmentent l'expression de la mPGES-1, tandis que la 15d-PGJ_2, un ligand de PPARg, diminue l'expression induite de la mPGES-1 par l'IL-1b dans les chondrocytes. Ces résultats suggèrent que la mPGES-1 est une cible thérapeutique intéressante pour contrôler la production de la PGE_2.

Mots clés :
Ostéoarthrite, Prostaglandine microsomale E synthase-1, Cartilage, Chondrocytes, PGE_2, PPARγ, 15d-PGJ_2.

TABLE OF CONTENTS

List of Figures

Introduction

Article

List of abbreviations

AA: Arachidonic acid

ADAMTS: A disintegrin and metalloprotease with thrombospondin motifs

AHR: Aryl hydrocarbon response element

AIA: Adjuvant-induced arthritis

ATF/CRE: Activating transcription factor/cyclic AMP response element

CAIA: Collagen antibody-induced arthritis

cAMP: Adenosine 3', 5'-cyclic monophosphate

C/EBP: CAAT enhancer-binding protein

CNS: Central nervous system

COX: Cyclooxygenase

CRE: cAMP responsive element

CREB: cAMP regulatory binding protein

CRTH2: Chemoattractant receptor-homologous molecule expressed on T helper (Th)2 Cells

DP: Prostaglandin D receptor

15d-PGJ_2: 15-deoxy-delta-12-14- PGJ_2

ECM: Extracellular matrix

EGF: Epidermal growth factor

EMSA: Electrophoretic mobility shift assay

FGF: Fibroblast growth factor

Egr-1: Early growth response -1

EIA: Enzyme Immunoassay

ELISA: Enzyme-Linked Immunosorbent Assay

EP: Eicosanoid receptor

FLAP: 5-lipoxygenase-activating protein

FGF: Fibroblast growth factor

GREs: Glucocorticoid response elements

GSH: Reduced glutathione

GST: Glutathione-S-transferase

HCG: Human chorionic gonadotrophin

HODE:	Hydroxy-octadecadienoic acid
Hsp90:	Heat shock protein 90
ICAM-1:	Intracellular adhesion molecule-1
IGF:	Insulin-like growth factor
iNOS:	Inducible nitric oxide synthase
KO mice:	Knock out mice
IL-1:	Interleukin-1
IL-1R:	Interleukin-1 receptor
LH:	Luteinizing (luteinising) hormone
LOX:	Lipoxygenase
LPS:	Lipopolysaccharide
LTA_4:	Leukotriene A_4
LTC_4:	Leukotriene C_4
MAPEG:	Membrane-associated proteins in eicosanoid and glutathione metabolism
MAPK:	Mitogen-activated protein kinase
MGST:	Microsomal glutathione S-transferase
MGST1-L1:	Microsomal glutathione S-transferase -1-like1
MMP:	Matrix metalloprotease
MT1-MMP:	Membrane type 1-MMP
NF-κB:	Nuclear factor κB
NO:	Nitric oxide
NSAID:	Nonsteroidal anti-inflammatory drugs
OA	Osteoarthritis
PDGF:	Platelet-derived growth factor
PGD_2:	Prostaglandin D_2
PGDS:	Prostaglandin D synthase
PGE_2:	Prostaglandin E_2
PGES:	Prostaglandin E synthase
$PGF_{2\alpha}$:	Prostaglandin $F_{2\alpha}$
PGG_2:	Prostaglandin G_2

PGH_2:	Prostaglandin H_2
PGI_2:	Prostaglandin I_2
PGJ_2 :	Prostaglandin J_2
PGs:	Prostaglandins
PLA_2:	Phospholipase A_2
PKA:	Protein kinase A
PPARs:	Peroxisome proliferator-activated receptors
PPARγ:	Peroxisome proliferative activated receptor, gamma
PPRE:	Peroxisome proliferator response element
RA:	Rheumatoid arthritis
RT-PCR:	Reverse transcription-polymerase chain reaction
RXR:	Retinoid X receptor
TGF:	Transforming growth factor
TIMP-1:	Tissue inhibitor of metalloproteinases
TNF-α:	Tumor necrosis factor α
TPA:	Tumor-promoting phorbol esters
TXA_2 :	Thromboxane A_2
TZD:	Thiazolidinediones
USF-1:	Upstream transcription factor 1
WT mice:	Wild type mice

Acknowledgement

This thesis is finished under Dr. Hassan Fahmi's supervision. Dr. Hassan Fahmi gave excellent suggestions during its proposal, experimental design, experimental procedure and thesis writing. I would like to thank him for all his helps.

I would like to thank Dr. Hassan Afif for his technical support during experimental procedure. I also would like to thank Saranette Cheng, Katherine Farrajota for their nice discussions and helps. I also thank Amal Bessissow for translating my summary into French.

I would like to thank my family for support, love and understanding during my period as a master student.

A. INTRODUCTION

I. Osteoarthritis (OA)

I.1. Definition and classification of OA

The term arthritis refers to many diseases, the most common of which is osteoarthritis (OA). OA is a group of overlapping distinct diseases, which may have different etiologies but with similar biologic, morphologic, and clinical outcomes. The disease processes not only affect the articular cartilage, but also involve the entire joint, including the subchondral bone, ligaments, capsule, synovial membrane, and periarticular muscle. Ultimately, the articular cartilage degenerates with fibrillation, fissures, ulceration, and full thickness loss of the joint surface. OA diseases are a result of both mechanical and biologic events that destabilize the normal coupling of degradation and synthesis of articular cartilage chondrocytes, extracellular matrix, and subchondral bone (Brandt et al, 2003). When clinically evident, OA diseases are characterized by joint pain, tenderness, limitation of movement, crepitus, occasional effusion, and variable degrees of inflammation without systemic effects (Brandt et al, 2003).

OA is classified into two groups: primary (idiopathic) and secondary. Primary OA is most common form and has no known cause, although it often is related to aging and heredity. Primary OA is divided into two forms: localized and generalized. The localized form affects single joint site (hands, feet, knee, hip, spine). The generalized form involves three or more joint groups. Secondary OA: An antecedent factor induces the disease, then the OA that follow is termed secondary. The factors include trauma, congenital or developmental diseases, metabolic diseases, endocrine diseases and other bone and joint diseases etc (Brandt et al, 2003).

I.2. Epidemiology of OA

I.2.1. Prevalence and incidence of OA

OA is an extremely common joint disorder in all population. Its high prevalence, especially in the elderly, and the frequency of OA-related physical

disability make OA one of the leading causes of disability in the elderly. OA of the hip and knee represents two of the most significant causes of adult pain and physical disability. It ranks fourth in health impact in women and eighth in men in the western world (Murray & Lopez, 1996). The physical and economic burden of OA is enormous, affecting up to 15% of the total population (>50% of the aging population over 60 years of age) (Poole et al, 2002).

OA has a higher prevalence, and more often exhibits a generalized distribution, in women than in men. Before the age of 50, men have a higher prevalence than women, but, after the age of 50 women have a higher prevalence, and this sex difference in prevalence further increases with age (Felson et al, 2000).

Overall, OA is the most common form of arthritis. It occurs frequently in knees, hands, hips, back, neck, spares wrists and ankles. The incidence and prevalence of this disease are higher in women than in men, especially after the age of 50. Many people have joint symptoms without X-ray change and vice versa.

I.2.2. Risk factors for OA

Risk factors for OA include systemic factors and local biomechanical factors (shown in Figure 1).

Systemic factors:

Age, Sex, and ethnicity: The most potent systemic vulnerabilities are increasing age and female gender. Disease incidence and prevalence increase dramatically with age. The Framingham study found that 27% of those aged 63 to 70 had radiographic evidence of knee OA, increasing to 44% in the over 80 age group (Felson et al, 1995). Racial factor is another systemic factor with those of Asian people having very low rates of hip OA (Haq et al, 2003).

Genetics: OA is a group of clinically heterogeneous disorders. Many genes have been linked to OA. There is most concordance with chromosomes 2q, 4 and 16. Families have been found with rare autosomal dominant patterns of inheritance of OA. The defective genes are often coding for structural proteins of the extracellular matrix (ECM) of the joint and collagen proteins (Haq et al, 2003).

Hormonal status: Some women after age 50 develop "menopausal arthritis" at the time of menopause. These gender and age related prevalence patterns are consistent with a role for post-menopausal hormone deficiency in increasing the risk of OA. Estrogen loss has been strongly implicated as a risk factor. Epidemiologic studies provide evidence that estrogen replacement therapy is associates with a reduction in the risk of knee and hip OA (Nevitt et al, 1994).

Nutritional factors: People in the lower vitamin C and vitamin D blood levels had a threefold risk of progression of knee OA (Felson et al, 1995). Vitamin C protects against damage by reactive oxygen species and it serves as a cofactor for enzymes contributing to type II collagen synthesis. Vitamin D sufficiency is necessary for active bone turnover which may be critical in OA (Brandt et al, 2003).

Local biomechanical factors:

Obesity: This is the strongest modifiable risk factor. Three to six times the body weight is transferred across the knee joint during walking. Any increase in weight should be multiplied by this factor to estimate the excess force across the knee joint when an overweight patient walks. Population-based studies show that overweight persons are at higher risk of OA than non-overweight control. In the Framingham study, women who lost an average of 11 lbs decreased their risk for knee OA by 50% (Felson et al, 1992).

Major joint injury: With a major joint injury, a person can sustain permanent damage of many of the structures within a joint. This damage alters the biomechanics of the joint, increases stress across particular areas of the joint and often dramatically increases the risk of OA. The Framingham study found men with a history of knee injuries had a relative risk of 3.5 for subsequent knee OA; for woman the relative risk was 2.2 (Felson, 1990). Prior joint surgery also is a risk factor in OA development.

Occupational and athletic activities: OA is common in those performing heavy physical work, especially if this involves knee bending, squatting, or kneeling. Dockers and miners have been found to have a higher prevalence of knee OA than those in sedentary jobs (Hunter et al, 2002). There is a significant relationship between occupational kneeling and repetitive use of joints during work and OA. Competitive

athletes are at greater risk for later development of OA. Epidemiologic study has demonstrated that participation in certain competitive sports increase the risk for OA (Buckwalter et al, 1997). Sports activities that appear to increase the risk for OA include those that demand high-intensity, acute, direct joint impact as a result of contact with other participants, playing surfaces, or equipment (Buckwalter et al, 1997). Repetitive joint impact and torsional loading also appear to be associated with joint degeneration.

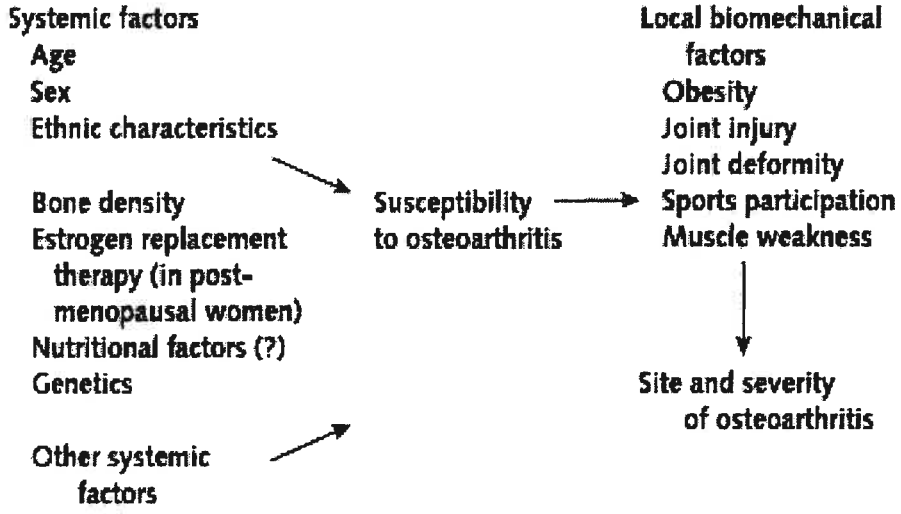


Figure1. Pathogenesis of OA with putative risk factors (Felson et al, 2000)

I.3. Articular cartilage

Cartilage is known as elastic cartilage, fibrocartilage or hyaline cartilage, depending on its different physical properties. Articular cartilage is a specialized avascular and neural connective tissue that provides covering for the osseous components of diarthrodial joints. It serves as a load-bearing material, absorbs impact, and is capable of sustaining shearing forces. The unique properties of this tissue are related to the composition and structure of its ECM, which is composed mainly of a high concentration of proteoglycans entangled in a dense network of collagen fibers and a large amount of water.

I.3.1.General structure of articular cartilage

Articular cartilage is organized in a manner that reflects the tensile and compressive force and shear stresses acting on this tissue. This tissue is composed of an extensive ECM synthesized by chondrocytes. It contains different zones with respect to depth from the articular surface and has a regional organization around the chondrocytes. The cartilage is classified into four zones: superficial zone, mid- zone, deep zone and calcified zone (Poole et al, 2001). (shown in Figure 2)

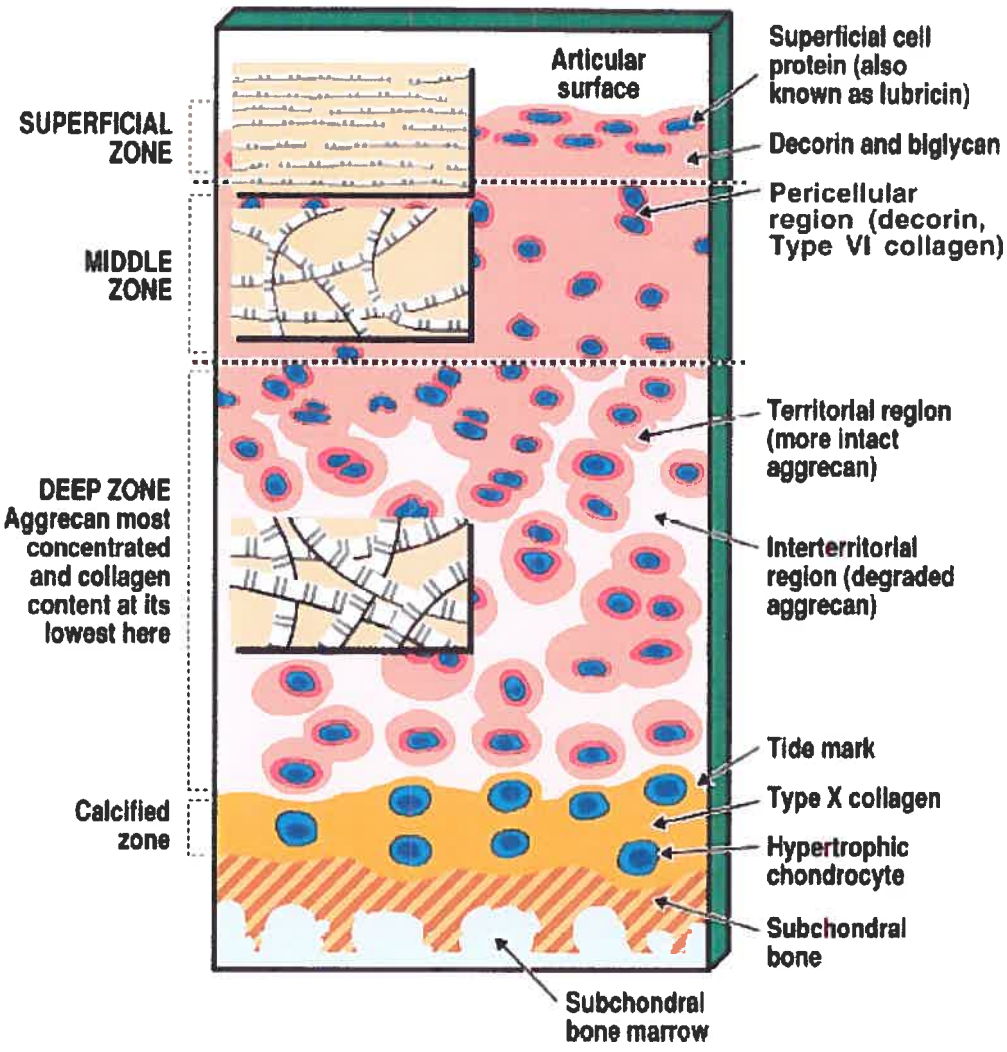


Figure 2. Diagrammatic representation of the general structure of human articular cartilage from an adult to show the zones, regions, and relationship with subchondral bone (Poole et al, 2001).

In the superficial zone the chondrocytes are flattened. The tissue in this region is maximally exposed to the shearing, compressive, and tensile forces of articulation. The collagen fibrils throughout the more superficial matrix are much thinner and are frequently arranged parallel to each other and to the articular surface. Here, the small proteoglycan decorin is most concentrated, being associated with the collagen fibrils, whereas the large proteoglycan aggrecan is present in its lowest concentration. Below the superficial zone is the mid-zone where cell density is lower. The mid-zone consists of rounded cells surrounded by an extensive ECM, rich in the proteoglycan aggrecan. In the deep zone, cell density is at its lowest but aggrecan content and fibril diameter are maximal, although collagen content is minimal. Cells in this zone are often grouped in clusters and resemble the hypertrophic chondrocytes of the growth plate. Adjacent to the deep zone is the calcified zone. The calcified cartilage zone separates the hyaline cartilage from subchondral bone. It appears to serve as an anchor of the cartilage to the bone as collagen fibrils from the radial zone penetrate into the calcified cartilage. In this zone, the cell population is very scarce and chondrocytes are usually smaller.

The cartilage matrix consists of distinct regions surrounding the chondrocytes of articular cartilage. All chondrocytes are surrounded by a thin pericellular matrix up to 2μm thick that contains few well-defined collagen fibrils, consists mainly of filamentous and fine fibrillar material. A territorial region surrounds this pericellular region that is present throughout the cartilage. In the deep zone, it is well-demarcated from the territorial region by differences in proteoglycan aggrecan structure and composition. This region is called the interterritorial region. It is the part of the matrix most remote from the chondrocytes. Degradation products of aggrecan probably are most concentrated here, produced as a result of incomplete proteolysis and retention of degradation products that retain binding for hyaluronan.

I.3.2. Composition of the articular cartilage

Condrocytes: Articular cartilage contains only one cell type, the chondrocyte. This occupies only approximately 2% of the total cartilage volume in human adults (Stockwell et al, 1979). The remainder is occupied by an extensive ECM that is synthesized by these cells. This contrasts to fetal and young immature (0–2 years) cartilages where cell volume is very much higher during growth. With increasing age, there is a progressive decrease in cell content and in matrix synthesis, the latter reaching its lowest point when the individual is 20 to 30 years (Stockwell et al, 1979). The chondrocytes are responsible for the metabolism of ECM.

ECM of cartilage: The ECM is composed of 65 to 80% water. The water content of cartilage plays an important role in maintaining the resiliency of the tissue and contributing to the nutrition and lubrication system. Collagen (mainly type II) accounts for about 15 to 25% of the wet weight. Its concentration is usually progressively reduced with increasing depth from the articular surface. It forms a fiber network that provides the shape and form of the tissue. The proteoglycan content (mainly the very large molecule called aggrecan) accounts for up to 10% of the wet weight. Aggrecan content increases with depth. It is responsible for the compressive properties associated with load bearing. The remainder of the matrix is normally accounted for by other collagens including V, VI IX, XI and XIV, link protein and a number of matrix proteins (Koopman et al, 1997).

Collagens: The structural backbone of cartilage matrix is the collagen fibril. It is composed mainly of type II collagen. It also contains type IX collagen and type XI collagen, both within and on the surface of the fibril, and as well leucine-rich proteoglycans, including decorin, fibromodulin, and biglycan (Koopman et al, 1997). Type II collagen that makes up the bulk of these fibers is specific for cartilage and is the primary collagen of articular cartilage. The fibrils that contain type II collage are composed of tropocollagen molecules (each of which contains a triple helix of three identical α chains), with nonhelical amino- and carboxyl-terminal telopeptide domains. Tropocollagen molecules assemble to form fibrils and larger fibers, stabilized by covalent interfibrillar cross-links. The most important mechanical properties of collagen fibres are tensile stiffness and strength.

Proteoglycan aggrecan: The major non-collagenous component in articular cartilage is proteoglycan aggrecan. It constitutes the second largest portion of solid phase in articular cartilage. This very large molecule consists of a central protein core of 2000 amino acids with several distinct domains and different functions. Its core protein contains three globular domains and two glycosaminoglycan-attachment domains. These domains play various roles to maintain cartilage structure and function. An N-terminal globular domain G1 contains a site for binding with hyaluronan and link protein to form huge aggregates. The link protein that has many structural features similar to the G1 domain of aggrecan stabilizes the complex. The G2 domain is homologous with the major part of the G1 domain. But it does not interact with HA. The C-terminal, G3 domains contain sequences homologous to the epidermal growth factor, complement regulatory component and a lectin and may be involved in interaction with other ECM glycoproteins. Other domains with important functional properties are the chondroitin sulfate domains, CS1 and CS2. They carry a very large number of negatively charged glycosaminoglycan chains of chondroitin sulfate. An extended protein domain next to the chondroitin sulphate region has a rather specific repeat structure and carries a number of keratan sulphate chains. Aggrecan provides the compressive stiffness of cartilage. This is achieved by hydration of the large numbers of chondroitin sulfate and keratan sulfate chains that occupy the core protein in the keratan sulfate and chondroitin sulfate rich regions between the G2 and G3 domains. Aggrecan creates a highly hydrated matrix, but the collagen fibrillar network limits hydration and swelling. Thus, aggrecan is only partially hydrated and exhibits a swelling pressure (Koopman et al, 1997). It is this property that endows cartilage with its compressive stiffness and ability to resist deformation and dissipate load.

I.4. Pathology of OA

I.4.1. Cartilage

The pathology of OA reflects both damage to the joint and reaction to the damage. The most striking gross changes are usually seen in the load-bearing areas of articular cartilage. In the earlier stages the cartilage is thicker than normal (Brandt et

al, 2003). Excess mechanical stress induces edema, with stretching and thinning of the superficial layer. Cartilage edema makes the perichondral collagen fibers more susceptible to deformation damage, resulting in increased apoptosis and necrosis of the vulnerable chondrocytes (Hashimoto et al, 1998). The reduction of the chondrocyte population decreases the capacity of the tissue to secrete and maintain matrix proteoglycans, initiating a cycle that accelerates the susceptibility to injury (Brandt et al, 2003). With disease progression, the joint surface thins and the proteoglycan concentration diminishes, leading to softening of the cartilage. The integrity of the surface is lost and vertical clefts develop (fibrillation). With joint motion the fibrillated cartilage is lost, exposing underlying bone. Areas of fibrocartilaginous repair may appear (Brandt et al, 2003). The chondrocytes replicate, forming clusters called clones. Later, the remaining cartilage becomes hypocellular.

I.4.2. Bone

While loss of articular cartilage represents the pathologic hallmark of OA, remodeling and hypertrophy of bone are also major features. At the beginning, activation of osteoclast-osteoblast system results in bone resorption and incremental bone formation. The remodeled bone matrix is more hydrated and less dense than bone more distant from the joint surface. Appositional bone growth occurs in the subchondral region, leading to the sclerosis; with decreased stress, bone resorption leads to osteoporosis (Brandt et al, 2003). Later in the OA process, with extensive erosion of the cartilage surface, trabecular microfractures may contribute to the stiffening of subchondral bone. Bone cysts form beneath the surface and weaken the osseous support for the overlying cartilage. Growth of cartilage and bone at the joint margins leads to osteophytes, which alter the contours of the joint and may restrict movement.

I.4.3. Synovium

The synovium consists of a single, discontinuous, intimal layer comprised of macrophages (type A cells) and fibroblasts (type B cells), embedded in connective

tissue containing thin collagen fibrils aligned parallel to the synovial surface. In early phases of OA, edema is within the synovium. As the edema fluid is resorbed, the matrix proteoglycan content increase. With the progression of OA, the synovial lining becomes more continuous as the intimal cells proliferate and as macrophages migrate into the tissue. In OA effusions, proteolytic enzymes secreted by the synovium act to digest cartilage matrix that has been sheared mechanically from the joint surface (Brandt et al, 2003).

I.5. Molecular mechanism of OA

I.5.1. Destruction of articular cartilage in OA

Cartilage loss is central to OA. The process of cartilage destruction in OA is basically an error in cartilage homeostasis. Normally, anabolic and catabolic pathways governing the synthesis and maintenance of ECM are in balance. While articular cartilage ECM protein turnover is quite modest under normal conditions, chondrocytes are able to synthesize and integrate into the ECM, those ECM proteins such as proteoglycans, collagen, fibronectin, integrins and other adhesive proteins which enable cartilage to maintain high tensile strength and low compressibility under load throughout the life-span of the individual. Chondrocytes function in response to cytokines and growth factor signals, and to direct physical stimuli, which interact in a complex manner. The end result is a change in the rate of synthesis versus that of enzymatic breakdown of the cartilage matrix, occurring both around the cells and at some distance. Both autocrine and paracrine actions have been demonstrated in chondrocyte and in synovial lining cells. In normal cartilage, there is strict regulation of matrix turnover: a delicate balance between synthesis and degradation. In OA, this balance is disturbed, with both degradation and synthesis usually enhanced. However, in OA, this equilibrium between anabolism and catabolism is weighted in favour of degradation.

Degradative proteinases, secreted by articular cartilage chondrocytes, such as matrix metalloproteinases (MMPs) play a major role in the degradation in OA. These include stromelysin-1 (MMP-3), gelatinases A (MMP-2), and B (MMP-9) and

collagenase-1 (MMP-1), collagenase-2 (MMP-8), collagenase-3 (MMP-13) and MT1-MMP (membrane type 1-MMP or collagenase 4 or MMP-14) (Shlopov et al, 1997). Most of these enzyme activities are increased in OA, whether by the mechanism of increased synthesis, increased activation of proenzymes by other MMPs or plasmin, or decreased inhibitor activity. In nearly all OA cells, MMP-3, MMP-8 and MMP-13 were elevated. Many of these MMPs are stimulated by exposure of the cells to inflammatory cytokines. To agonize the effects of MMPs, expression levels of inhibitors such as tissue inhibitor of metalloproteinases (TIMP)-1 are reduced in OA.

In OA, collagenase is responsible for the breakdown of collagen type II scaffolding in cartilage. All three collagenases, collagenase-1 (MMP-1), collagenase-2 (MMP-8), collagenase-3 (MMP-13) cleaves type II collagen. Collagenase-3 is the enzyme responsible for most of the collagen degradation (Billinghurst et al, 1997), which plays the greatest part in the pathology of OA degrading the resident collagen fibrils more remote from the cell in the territorial and interterritiorial matrix. This collagenase is also used to remodel matrix in the growth plate. Collagenase-1 is believed to be more involved in the degradation of newly synthesized collagen. The activities of collagenases are clearly increased in both advanced and end stage OA and in the early development of focal OA lesions. In addition, stromelysin-1 (MMP-3) can cleave in the nonhelical telopeptide of type II and IX collagens (Wu et al, 1991), leading to disruption of a collagen crosslink. This cleavage could result in a disrupted fibril structure. Furthermore, type II collagen telopeptide can also be cleaved by MMPs 7, 9, 13 and 14. These findings indicated the presence in OA of a host of enzyme candidates capable of disrupting the collagen network. Disruption of this network will eventually lead to destabilization of the joint.

The large proteoglycan aggrecan, is cleaved by different MMPs and is also degraded by a special class of MMPs known as aggrecanases at distinct sites in the core protein. Two aggrecanases (aggrecanase-1 and aggrecanase-2) have been identified as part of the ADAMTS family. These proteinases selectively cleave particular peptide linkages in the G1-G2 interglobular domain and are largely responsible for the turnover of aggrecan in articular cartilage of both normal and OA joints. There is evidence for the activities of both types of proteinases in OA. Analysis

of the proteoglycan aggrecan have revealed that excessive cleavage occurs in OA cartilage in the core protein, particularly in the interglobular domain between the G1 and G2 domains (Poole, 1999).

After the initial cleavage of type II collagen by collagenases it is denatured and lost. Chondrocyte subsequently undergo further phenotypic change becoming hypertrophic and expressing and secreting type X collagen. This differentiation is normally seen in the growth plates as part of endochondral ossification (Poole et al, 2001). Calcification of articular matrix also occurs in OA in association with these changes. Thus, chondrocyte differentiation in OA seems to be a response to extensive damage to the collage fubrillar network. Moreover, hypertrophic cells eventually undergo apoptosis. This is commonly seen in OA cartilage.

I.5.2. Mechanism responsible for matrix destruction and disease progression in OA

During matrix degradation, excessive catabolism of articular cartilage results in the release into synovial fluid of matrix breakdown products including chondroitin sulfate and keratan sulfate peptides, PG fragments, type II collagen peptides, fibronectin fragments, chondrocyte membranes, etc. all of which are antigenic and elict an inflammatory response in the synovial membrane (Smith et al, 1997). The activated synovial macrophages in the membrane release cytokines (IL-1, TNF-α, etc), PGE_2, proteinases and oxygen free radicals (superoxide, nitric oxide (NO)) into adjacent tissues and the synovial fluid. These mediators in turn can act on chondrocytes and synovial fibroblasts, modifying their biosynthesis of PGs, collagen and hyaluronan as well as promoting release of catabolic mediators. Some of the matrix breakdown products are known to induce the expression and secretion of MMPs and prodegradative cytokines such as IL-1 and TNF-α. There is increased expression in OA chondrocyte of the IL-1 and its receptors (Melchiorri et al, 1998; Martel-Pelletier et al, 1992). TNF-α is also upregulated in OA (Melchiorri et al, 1998) and TNF-α receptors show increased expression when compared to normal cartilage (Webb et al, 1997). These cytokines derived from chondrocytes and the synovial lining play a key role in cartilage degeneration in OA. Cytokines are responsible for

accelerating the destruction of cartilage ECM via their ability to up-regulate metalloproteinase gene expression. They also serve to suppress compensatory ECM protein biosynthesis by chondrocytes. Another pathway involving the induction of NO in cartilage by cytokines appears relevant to programmed cell death (apoptosis) and OA pathology (Lotz, 1999).

While cytokines are clearly important in up-regulating MMP gene expression, other pathways relevant to the process include potent biological activity of fibronectin fragments. Fibronectin fragments enhance levels of catabolic cytokines and also up-regulate MMP expression, significantly enhance loss of proteoglycans from cartilage and transiently suppress proteoglycans synthesis (Malemud, 1999). Changes in ECM loading can also induce ECM cleavage as well as changing the synthesis of ECM macromolecules (Poole, 1999). The pathological changes in cartilage ECM in OA are likely to result in a disturbance of the normal balance between mechanical loading and direct cytokine/growth factor signalling changing gene expression. Figure 3 summarizes some metabolic pathways responsible for cartilage degradation in OA joint.

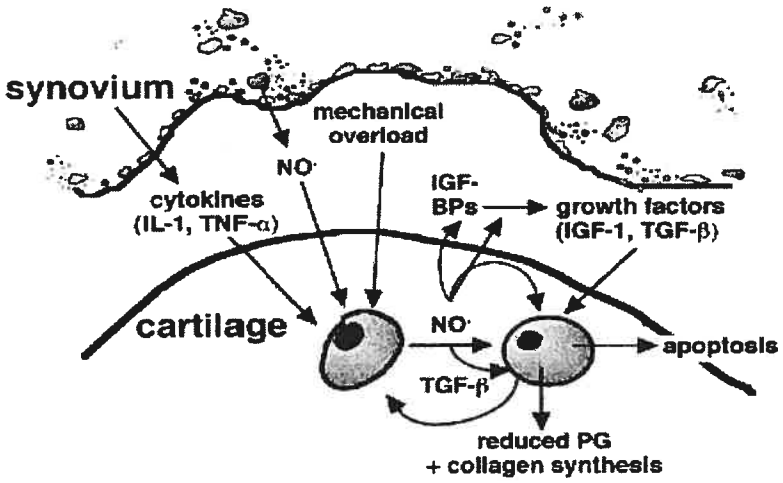


Figure 3. Summary of some the metabolic pathway responsible for cartilage degradation in OA joint (Studer et al, 2000).

II. Prostaglandins biosynthesis pathway

II.1.Biosynthesis of eicosanoids:

II.1.1. Eicosanoids

The term eicosanoid refers to any twenty-carbon (C20) fatty acid. Prostaglandins, thromboxanes, leukotriene and lipoxins are related compounds known as eicosanoids, which have a large variety of biological activities. Most eicosanoids are biosynthesized from C20 polyunsaturated fatty acids, primarily arachidonic acid (AA). AA is the most plentiful C20 polyunsaturated fatty acid in most mammals (Zubay, 1988).

II.1.2. Release of AA and phospholipase A_2 (PLA_2) enzymes

AA is a 20-carbon unsaturated fatty acid distributed throughout the lipid bilayer of all mammalians. It is derived directly from the diet or via modification of linoleic acid, and normally is stored in the cell membranes, esterified in the sn-2 position of phospholipids (Irvine, 1982). Under normal conditions, the level of free AA is low, but upon stimulation, AA is released by the hydrolytic action of PLA_2 enzymes (involving secretory, cytoplasmic or both types of PLA_2. AA is metabolized through oxygenation by three enzymatic pathways in mammals. Through cyclooxygenase (COX) pathway, AA is converted to prostaglandin H_2 (PGH_2). PGH_2 is then converted to PGE_2, $PGF_{2\alpha}$, TXA_2, PGD_2, or PGL_2. Through the 5-lipoxygenase (LOX) pathway, AA can be converted to a leukotriene A_4 (LTA_4). LTA_4 can be further metabolized into various leukotrienes and monooxygenase pathway leads to a series of epoxy-and hydroxy-acid derivatives. Many PLA_2 enzymes are active within the cell or in the close vicinity and have distinct, but interconnected roles in AA release. At least 19 PLA_2 enzymes have been indentified in mammal, amongst which the cytosolic PLA_2 ($cPLA_2$), secretory PLA_2 ($sPLA_2$) and Ca2+-independent PLA_2 ($iPLA_2$) families have been implicated in eicosanoid production (Kudo, 2002).

II.2. Prostaglandins biosynthesis

Prostaglandins are small lipophilic molecules that are produced by a variety of cell types in response to both physiological and pathological stimuli. The general pathway for the biosynthesis of prostaglandins is illustrated in figure 4. The first step

in the pathway for the biosynthesis of prostaglandins involves intracellular release of AA from plasma membrane phospholipids via the action of PLA_2. AA is then converted sequentially to PGG_2 and PGH_2 by the COX and peroxidase activities of a single enzyme, PGH synthase (also called COX). There are two forms of PGH synthase, a constitutive form (COX-1) and an inducible form (COX-2). Different terminal synthases then convert PGH_2 to the 5 primary prostaglandins: thromboxane A_2 (TXA_2), PGD_2, PGE_2, $PGF_{2\alpha}$, or prostacyclin (PGI_2). PGD_2 gives rise to the important derivatives 9α, 11β PGF_2 and the J-series PGs including PGJ_2, Δ^{12}PGJ2 and 15d-PGJ_2, the latter through a series of non-enzymatic steps. Additional active and inactive prostaglandins also derive from further isomerization of PGE_2, $PGF_{2\alpha}$, PGJ_2 and TXA_2. The resulting products then exit the cell via a carrier mediated process to activate G protein-linked prostanoid receptors or in some cases may interact with nuclear receptors.

Physiological actions of $PGF_{2\alpha}$, PGI_2 TXA_2 , PGD_2 and PGE_2 series prostaglandins are mediated by binding to specific high affinity G-protein coupled cell surface prostanoid receptors.There is one PGF receptor termed FP, one PGI receptor termed IP, one TXA receptor termed TP receptors, and two PGD receptor termed DP and CRTH2. PGE has 4 separate receptors, termed EP_1-EP_4, each encoded by a distinct gene. Depending on the receptor, the consequence of ligand binding to these receptors can be increased cyclic AMP, decreased cyclic AMP, or a phosphoinositide response (Narumiya, 1995). In addition to plasma membrane receptors, recent evidence shows that prostanoids also can bind and signal through nuclear hormone receptors, PPARs. Three distinct PPAR isoforms- PPARα, β/δ and γ- have been isolated and characterized. PPARγ binds some AA metabolites, especially the PGD_2 metabolites such as 15d-PGJ_2. PPARα and δ bind a stable analog of PGI_2, carbaprostacyclin (cPGI).

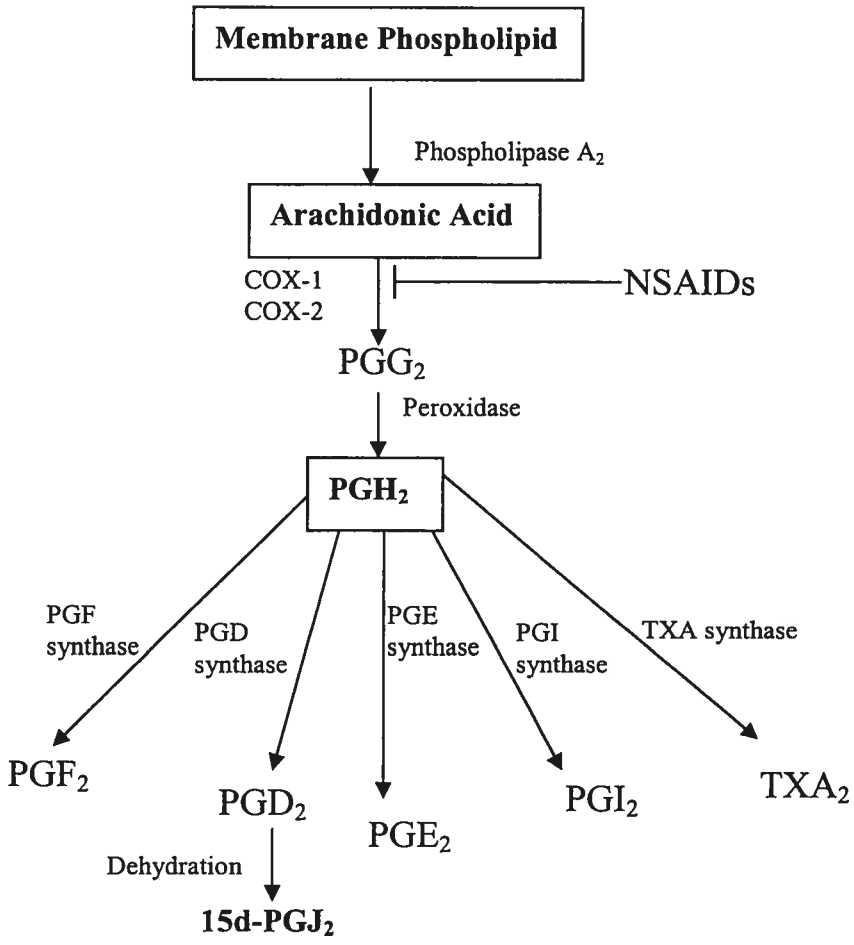


Figure 4. General pathway for synthesis of prostaglandins

The nature of the final active product depends on the cell type, the stimulus, and the presence of distinct PG synthase. TXA_2 synthase (TXAS) is present in a platelets and macrophages, prostacyclin synthase (PGIS) is present in the uterus, two type of PGD_2 synthase (PGDS) are found in brain and mast cells, and enzymes responsible for the isomerization of PGH_2 to PGE_2 are expressed in synovial fibroblasts (Stichtenoth et al, 2001).

Prostaglandins play critical roles in numerous biological prosesses, including kidney development, reproduction, bone metabolism, inflammation, maintenance of gastrointestinal integrity, angiogenesis, modulation of immune responses, apoptosis and mitogenesis. In contrast to some homones, which are released from a specific site but have broad systemic effects in distant organs, prostaglandins are synthesized in broad range of tissue type and serve as autocrine or paracrine mediators to signal changes within the immediate environment.

PGE_2 produced is released from the cells and act on four types of the PGE receptors, EP_1, EP_2, EP_3 and EP_4, all of which are coupled with the trimeric G-protein signalling. PGE_2 plays crucial roles in various biological events, such as neuronal functions, female reproduction, vascular hypertension, tumorigenesis, fever, gastric mucosal protection, pain hypersensitivity, anti-allergic response and inflammation-associated bone resoption.

Overproduction of PGE_2 is often associated with various diseases. Elevated production of PGE_2 plays an important role in the pathogenesis of arthritis. Several studies suggest that PGE_2 is the major prostaglandin (PG) produced by articular joint cells and is involved in inflammation, apoptosis, angiogenesis, and tissue degradation that characterize arthritic diseases. The induction of cartilage degradation by PGE_2 is due to the inhibition of collagen synthesis, induction of MMPs production and induction of chondrocyte apoptosis. PGE_2 is largely produced in arthritic joint tissues and excessive production of PGE_2 has been reported in serum and synovial fluids of rheumatoid arthritic and osteoarthritic patients. Treatment with neutralizing anti-PGE_2 antibodies prevents acute and chronic inflammation in a rat adjuvant arthritis model (Portanova et al, 1996). Mice lacking COX-2 or PGE_2 receptors display reduced incidence and severity of collagen-induced arthritis. These animals showed reduced

inflammation and less cartilage and bone destruction (Myers et al, 2000). The role of PGE_2 in arthritis is also supported by effective suppression of pain and inflammatory responses in arthritis by nonsteroidal antiinflammatory drugs (NSAIDs) that reduce PGE_2 biosynthesis (Crofford, 2002).

II.3. COXs

COX, also called PGH synthase, is a heme-containing enzyme that catalyzes the first two steps in the biosynthesis of the prostaglandins from the substrate AA. Two sequential enzymatic reactions are the bis-oxygenation of AA leading to production of PGG_2 (COX reaction) and reduction of 15-hydroperoxid of PGG_2 leading to formation of PGH_2 (hydroperoxidase reaction). Three COX isoforms, COX-1, COX-2 and COX-3, are found in mammals. COX enzyme was first purified in 1976 and cloned in 1988 (Merlie et al, 1988). In the early 1990s, COX was demonstrated to exist as two distinct isoforms. COX-1 is constitutively expressed as a "housekeeping" enzyme in most tissues. COX-2 is not constitutively expressed in appreciable amounts by most normal tissues, but is rapidly induced by proinflammatory cytokines, tumor promoters, oncogenes, and growth factors. COX-3 was recently identified and shown to exhibit the catalytic features of COX-1 and COX-2. COX-1 and COX-2 have very different expression profiles in several physiological processes. The COX isozymes are also involved in pathological processes. COX-1 is involved in thrombosis, while COX-2 mainly involved in inflammation, pain, fever, angiogenesis, cancer, Alzheimer's disease and several forms of arthritis. COX-1 and COX-2 are of particular interest because they also are the major targets of NSAIDs including aspirin, ibuprofen, and the new COX-2 inhibitors.

II.3.1. Gene structures and expression of COX isoforms

COX-1 and COX-2 have a molecular weight of 71 KDa and are almost identical in length. The COX monomer consists of three structural domains: **(a)** An N-terminal epidermal growth factor (EGF)-like domain of 50 amino acids at the N terminus. The EGF-like domain may play a role in the integration of maturating COX into the lipid bilayer. **(b)** A membrane-binding domain of about 50 amino acids. The membrane-binding domain contains four short, consecutive and amphipathic α-

helices. This creates a hydrophobic surface that would interact with the one face of the lipid bilayer, allowing COX enzymes to integrate into membranes through the monotopic mechanism (Picot et al, 1994). **(c)** A large C-terminal globular catalytic domain (about 460 amino acids) with a heme-bonding site. This domain is almost entirely comprised of α-helical structure, shares a great deal of structural similarity to myeloperoxidase. The C-terminal PTEL and STEL sequences in COX-1 and COX-2, respectively, represent an ER retention signal. The major sequence differences between COXs isoforms occur in the membrane binding domain (Spencer et al, 1999). A unique difference between COX-1 and 2 is 18 amino acids inserted 6 residues in from the C terminus of COX-2 that are not present in COX-1. Mature, processed COX-1 contains 576 amino acids; the mature form of COX-2 contains 587 amino acids. There is a 60%-65% sequence identity between COX-1 and 2 from same species and 85%-90% identity among individual isoforms from different species. However, the gene for COX-1 is approximately 22 kb in length with 11 exons and is transcribed as a 2.8 kb mRNA, whereas that for COX-2 is approximately 8.3 kb in length with 10 exons and is transcribed as 4.4 kb mRNA (Tanabe & Tohnai, 2002). The COX-1 and COX-2 genes map to human chromosomes 9q32-q33.3 and 1q25.2-q25.3, respectively (Tanabe & Tohnai, 2002)

COX-1 and COX-2 have significant sequence homology and identical catalytic activity, but their expression pattern is markedly different. COX-1 has been found in nearly all tissues under basal conditions and is thought to play a 'housekeeping' role. Nevertheless, COX-1 is preferentially expressed at high level in selected cells and tissues, including endothelium, monocytes, platelets, renal collecting tubules, and seminal vesicles, indicating that it is developmentally regulated (Smith et al, 2000). In contrast to COX-1, levels of COX-2 are typically low or absent in most tissues. However, COX-2 can be induced by several physiological and proinflammatory stimuli, including IL-1, TNF-α, lipopolysaccharides (LPS), transforming growth factor (TGF)-β, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) and hormones in many cell types like macrophages, monocytes, synoviocytes, chondrocytes, osteoblasts, leukocytes and endothelial cells (Dubois et al, 1998). The induction of COX-2 is usually transient, with a return to base

line within 24-48 hours (Williams et al, 1999). COX-2 is expressed constitutively in the brain, kidney, during ovulation and blastocyst implantation.

II.3.2. Regulation of COX-2 expression

The COX-1 promoter region lacks a functional TATA or CAAT box and is GC-rich, which is consistent with a housekeeping gene. There are several putative transcriptional regulatory elements in the promoter region of the COX-1 gene, such as two Sp-1 sites, two AP-2 sites, NF-IL-6 site and GATA. The two Sp1 sites contribute to constitutive expression of COX-1 (Xu et al, 1997). To date these Sp1 sites are the only cis-acting elements documented to regulate transcription of COX-1.

The promoter of the COX-2 gene contains a TATA box and various transcription elements, such as NF-IL-6, AP-2, Sp1, NF-kB, CRE and E-box. So far, only a limited class of elements are shown to be involved in the regulation of COX-2 transcription, often in synergy, such as E-box, ATF/CRE sequences, NF-IL6 CAAT enhancer binding sequence(C/EBP) and two NF-kB binding sites. The transcription factors that bind and activate COX-2 transcription involved C/EBPβ and C/EBPα for the NF-IL-6 elements, AP-1, ATF and CREB for the CRE elements, and USF-1 for the E-box. Dependence on NF-kB signalling for COX-2 expression has been demonstrated by use of pharmacologic inhibitors of IkB kinase (Gallois et al, 1998).

The signalling pathways that mediate COX-2 expression are tissue-specific and depend on the stimulus. A number of signalling pathways are likely to regulate transcription of COX-2. These include NF-kB and C/EBP, two common signalling pathways in inflammatory response, and three mitogen-activated protein kinase (MAPK) signalling cascades, ERK1/2, JNK/SAPK, and p38. Each of these signalling pathways has been shown to contribute or be solely required for increased expression of COX-2 in one or more cultured cell systems.

The MAPK cascade is a very important signalling pathway for COX-2 expression and consists of three different subgroup of kinase (ERK: extracellular-regulated kinase, JNK/SAPK: Jun N-terminal kinase/stress activated protein kinase, and p38: p38 mitogen-activated protein kinase). The ERKs are mainly activated by growth factors and oncogenes including v-src and v-ras. Cellular responses to mitogens are generally mediated by sequential activation of receptor tyrosine kinases, Src, Ras, and

one or more of the MAPK pathways. Expression of COX-2 following stimulation with serum and PDGF, or in response to v-Src or Ha-Rasexpression, requires activation of the ERK1/2 and JNK/SAPK pathways (Xie & Herschman, 1995; Sheng et al, 1998). The COX-2 gene has been shown to be an important Ras target since oncogenic mutation in Ras and overexpression of COX-2 is found in many forms of human cancers, including breast cancer and colorectal carcinoma (Subbaramaiah et al, 1996).

The JNK/SAPK and p38 pathways are activated by inflammatory stimuli, including IL-1β, TNF-α, and LPS, as well as the phorbol ester TPA and environmental stress, like oxidative stress. Proinflammatory cytokines including TNF-α and IL-1β have been shown to selectively activate JNK and p38 MAP kinase in cultured human articular chondrocytes (Geng et al, 1996). Several studies demonstrated that the selective p38 MAPK inhibitors prevented IL-1β-induced COX-2 expression in human synovial fibroblasts (Faour et al, 2001) and chondrocyte cell line (Thomas et al, 2002).

The NF-κB pathway is a common mediator of inflammatory responses and plays an important role in COX-2 expression in several cell types, including rheumatoid synoviocytes (Crofford et al, 1997). The COX-2 promoter contains two consensus sequences for the cis-acting regulatory sequences that are recognized by the NF-κB family of transcription factors. IL-1β treatment of human synovial fibroblast induced binding of the p65-p50 heterodimer and the p50 homodimer to the COX-2 promoter, and pre-treatment of the cells with NF-κB p65 antisense oligonucleotides prevented NF-κB binding and markedly decreased COX-2 expression (Crofford et al, 1997). Furthermore, transfection experiments with reporter plasmid demonstrated that mutations in the NF-κB cis-regulatory sites attenuate transcriptional activation of the COX-2 promoter in response to TNF-α stimulation (Yamamoto et al, 1995). NF-κB regulates COX-2 expression in response to the appropriate activators in specific cell types.

The C/EBPβ and C/EBPδ transcription factors are commonly involved in the regulation of inflammatory responses. This family of transcription factors is activated by most of the inflammatory stimuli that induce COX-2 expression. An NF/IL-6 regulatory element is present in the COX-2 promoters from all species examined and C/EBP proteins have been shown to bind to these promoter sequences. In human

synovial fibroblasts, TNF-α induced c/EBP binding to COX-2 promoter in addition to NF-κB (Alaaeddine et al, 1999). The C/EBP transcription proteins appear not to work independently but instead to cooperate with USF-1, NF-κB and c-jun to activate COX-2 transcription (Morris & Richards, 1996).

Dexamethasone is a common anti-inflammatory steroid, which binds to the glucocorticoid receptor and activates transcription of a number of genes via glucocorticoid response elements (GREs). Dexamethasone is an efficient suppressor of inflammatory-induced COX-2 expression although the COX-2 promoter does not contain GREs. The mechanism for glucocorticoid-mediated repression of COX-2 induction involves suppression of the AP-1 and NF-κB-dependent transcription (Scheinman et al, 1995; Yang-Yen et al, 1990) and destabilization and degradation of COX-2 mRNA and protein (Newton et al, 1998). The nuclear receptor PPARγ has been shown to down-regulate the expression of COX-2. PPARγ regulate gene expression by binding their heterodimeric partner retinoid X receptor to specific PPAR-responsive elements (PPREs). The promoter region of the human COX-2 gene harbors a PPRE at –3721 to –3707 bp (Meade et al, 1999). 15d-PGJ_2, a PPARγ ligand, covalently binds to IκB kinase, leading to inactivation of the NF-κB pathway and thereby to repression of COX-2 transcription (Straus et al, 2000). On the contrary, other studies have shown that PPARγ augments COX-2 transcription by binding to the PPRE element in the COX-2 promoter (Meade et al, 1999).

II.3.3. Biological function of COX-2

COX-2 and COX-1 have significant differences in tissue expression and regulation. Therefore they have different biological function. COX-2 is associated with inflammation and many malfunctions.

COX-2 has some role in regulating brain function. High basal levels of COX-2 are found in the brain. In the central nervous system (CNS), COX-2 is up regulated by neural activity. COX-2 protein or mRNA was detected in neurones and in the nonneuronal cells of the CNS (Yamagata et al, 1993). These suggested that COX-2 enzyme might be involved in CNS function. Proinflammatory cytokine IL-1 β was shown to be the major inducer of COX-2 up-regulation in the CNS. Intraspinal

administration of an interleukin-converting enzyme or COX-2 inhibitor decreased inflammation-induced central PGE_2 levels and mechanical hyperalgesia. These indicated that IL-1 β-mediated induction of COX-2 in the CNS contributed to inflammatory pain hypersensitivity (Samad et al, 2001). Fever is thought to be the effect of PGE_2 in CNS. COX-2-knockout mice suppressed both fever and PGE_2 level in the CNS (Li et al, 1999), implying that PGE_2 involved in the febrile response may drive from COX-2.

Prostaglandins (PGs) are involved in normal renal function including control of renin release, control of tubular function and regulation of vascular tone. In COX-2 null mice, the kidneys fail to develop normally resulting in death, whereas COX-1 null mice fail to produce a detectable renal pathology (Morham et al, 1995). Additionally, the involvement of COX-2 in renal functions was also suggested by clinical studies. Clinical studies showed that the COX-2 inhibitors, similar to other NSAIDs, cause qualitative changes in urinary prostaglandin excretion, glomerular filtration rate, and sodium retention. (Brater et al, 2001). Thus, COX-2 may play a role in physiological renal functions.

Ovulation, the process by which oocytes are released from the preovulation follicle in the ovary, is accompanied by induction of prostaglandin synthesis as a consequence of the LH surge. This marked response led to the first observation of COX-2 induction during a normal physiological event. The induction of COX-2 is necessary for the successful rupture of the follicle, probably mediating directly the generation or activation of proteolytic enzymes necessary for this process (Tsafriri, 1995). After fertilization, COX-2 appears to mediate the embryo-uterine interactions during implantation. COX-2 null mice show multiple failures in reproduction function, including ovulation, fertilization, implantation, and decidualization, underscoring the multiple roles of PGs during these processes (Lim et al, 1997).

Inflammation and arthritis: Studies using animal models of inflammation arthritis have provided evidence that increased expression of COX-2 is responsible for increased prostaglandin production seen in inflamed joint tissues (Anderson et al, 1996). COX-2 induction has been observed in human OA-affected cartilage (Amin et al, 1997). Other studies found that COX-2, not COX-1, expression was elevated in a disease-related pattern in synovial tissues from patients with rheumatoid arthritis (RA),

ankylosing spondylitis, psoriatic arthritis, and OA (Siegle et al, 1998). The proinflammatory agents IL-1, TNF-α and LPS, as well as the growth factors TGF-β, EGF, PDGF, and FGF, have all been shown to induce COX-2 expression in primary culture cells derived from human synovial tissue or catilage. On the other hand, the anti-inflammatory cytokines IL-4 and IL-13, as well as the immunosuppressive glucocorticoids, are shown to decrease COX-2 levels (Crofford, 1997).

COX-2 enzymes also play functional roles in tumorigenesis. High levels of constitutive expression of COX-2 have been found in various cancer cells and tissues, and studies employing overexpression, antisense suppression, and specific inhibitors of COX-2 have demonstrated that COX-2 contributes to the progression of several types of cancer. Both human and animal colorectal tumors express high levels of COX-2 (Eberhart et al, 1994). There is a reduction in the relative risk of colorectal cancer in individuals taking NSAIDs (Marnett, 1992). Suppression of tumorignesis in COX-2 (-/-) mice has confirmed epidemiological studies, demonstrating that NSAIDs suppress the incidence of colon cancer (Oshima et al, 1996). COX-2 may blunt the apoptotic response in tumor cells and may play a role in the regulation of angiogenesis associated with neoplastic tumour cells (Tsujii et al, 1998).

The cytoprotective actions of prostaglandin preventing gastric ulceration are mediated by endogenous prostacyclin and PGE_2, which reduce gastric acid production, stimulate gastric fluid secretion, increase secretion of viscous mucus and exert a direct vasodilator action on gastric mucosa. Classical NSAID use causes a variety of problems in the gastrointestinal tract, including irritation and ulceration of the stomach lining. The primary mechanism of NSAIDs in the treatment of inflammation is the inhibition of both COX-1 and COX-2. COX-2 mediates the inflammatory response. An NSAID that inhibits COX-2 selectively should decrease inflammation but not influence normal physiologic functions and thus should cause fewer gastrointestinal side effects. Selective COX-2 inhibitors are widely used. Rofecoxib and the novel COX-2 inhibitors etoricoxib and valdecoxib have a higher degree of COX-2 selectivity than traditional NSAIDs. However, rofecoxib induces thromboembolic adverse effects more frequently than classical NSAIDs. Caution is warranted regarding the use of these drugs (Evensen et al, 2005).

II.4. PGE Synthase (PGES)

II.4.1. The MAPEG-supperfamily

A widespread superfamily MAPEG (membrane-associated proteins in eicosanoid and glutathione metabolism) has been defined according to enzymatic activities, sequence motifs, and structural properties. A total of 136 proteins belonging to the MAPEG superfamily were found in database and genome screenings. All MAPEG proteins have similar molecular masses of 16-18 kDa and, except 5-lipoxygenase-activating protein (FLAP). All MAPEG proteins have similar three dimensional and membrane-spanning topographic properties (Jakobsson et al, 2000). Multiple sequence alignments of human MAPEG members reveal six strictly conserved amino acids. The family consists of six human proteins including FLAP, leukotriene C_4 (LTC_4) synthase, microsomal glutathione *S*-transferase 1 (MGST1), MGST2, MGST3, and MGST1-like 1 (MGST1-L1 or PGES). The genes encoding these proteins all reside on different chromosomes. In addition, several nonmammalian members have been identified, including those from plants (*Arabidopsis thaliana*, *Oryza sativa*, and *Ricinus communis*), fungi (Aspergillus nidulans), and bacteria (Synechocystis sp. [SynMGST], Escherichia coli, and Vibrio cholerae)(Jakobsson et al, 2000).

On the basis of the multiple sequence alignments, MAPEG family can be subdivided into four subgroups. The first subfamily consists of the members FLAP, LTC_4 synthase, and MGST2 and is important for leukotriene biosynthesis. The second subfamily consists of MGST3 together with the members found in plants and fungi. The third subfamily is composed of the proteins identified in bacteria (*E. coli* and *V. cholerae*). The human MGST1 and MGST1-L1 proteins constitute a fourth subgroup possibly involved in cytoprotection. (Jakobsson et al, 2000).

II.4.2. PGES identification

Metabolism of AA by COX yields only the unstable intermediary PGH_2, which can be further metabolized into PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2, or TXA_2. The enzyme responsible for the isomerization of PGH_2 into PGE_2 is PGES, the terminal enzyme responsible for PGE_2 synthesis. PGES activity, in most cases glutathione (GSH)-dependent, has been detected both in microsomal and cytosolic fractions of various cells, more than one form of PGES exist. At least four distinct PGES isoforms have

been identified, including cytosolic PGES (cPGES), GSTμ, microsomal PGES-1 (mPGES-1), and mPGES-2.

Human mPGES-1 is a member of the MAPEG superfamily. It was initially discovered and identified as a homologue of MGST1 with 38% identity on the amino acid sequence level. The protein thus was referred to as MGST1-like 1(MGST-L1). The same protein was also identified as a p53-induced gene and referred to as PIG12. In 1999, Jakobsson et al first reported mPGES-1 (Jakobsson et al, 1999). The recombinant human microsomal GST-l-like 1 (MGST1-L1) has an ability to catalyze the coversion of PGH_2 to PGE_2 with strict substrate specificity. Then this protein was cloned from several animal species and shown to represent a long-sought membrane bound form of PGES. This enzyme now is called mPGES-1. Soon after mPGES discovery, two cytosolic forms of PGES were purified and identified. One enzyme termed cytosolic PGES (cPGES). cPGES is a 23 kDa cytosolic protein and identical to p23, a heat shock protein 90 (Hsp90)-associated protein (Tanioka et al, 2000). The other is a member of the μ form of the cytosolic GST family. In 2002, the second form of membrane-associated PGES, termed mPGES-2 was identified.

II.4.3. mPGES-1 gene structure and catalytic function:

The primary structures of mPGES-1 proteins of various animal species reveal a high degree of sequence homology (≥80%). mPGES-1 also shows significant homology with other MAPEG superfamily proteins, including MGST-1, MGST-2, MGST-3, FLAP and LTCS, with the highest homology being found with MGST-1 (~40%) (Jakobsson et al, 1999).

The gene for human mPGES-1 maps to chromosome 9q34.3 is divided into three exons and two introns, and span 14.8 kb. Exon-intron junctions follow the GT-AG rule except for the 5'-site of intron 2, which consists of GC instead of GT. The intron sizes are 4.2 kb and 8.8 kb respectively (Forsberg et al, 2000). The gene structure of mPGES is similar to its closest relative MGST1 with regards to exon/intron borders and differs from other MAPEG members that contain additional exons. The cDNA for human mPGES-1 encodes a protein composed of 152 amino acid residues (~16 kDa). Mutation of Arg110, in mPGES-1, which is the residue strictly conserved in all

MAPEG proteins, abrogates its catalytic activity, indicating an essential role of this residue (Murakami et al, 2000). The cofactor GSH is absolutely required for mPGES-1 enzymatic activity. GSH involves in detoxification reactions with hydrogen peroxide and organic peroxides and also has a stabilizing effect on solubilized mPGES-1.

mPGES-1 activity was inhibited by the COX-2 inhibitory NSAID NS-398 and sulindac sulfide with IC_{50} values of 20,80 μM. mPGES-1 was also inhibited by MK-866, an inhibitor of FLAP and LTC_4 with IC_{50} values of 1-5 μM (Mancini et al, 2001). MK-866 binds to the AA-binding region of FLAP, which is highly conserved in LTC_4 and mPGES-1 and could possibly be involved in the binding of eicosanoids (Mancini et al, 2001). Furthermore, 15d-PGJ_2 and some polyunsaturated fatty acids were also reported to inhibit the activity of mPGES-1 (Quraishi et al, 2002). mPGES-1 activity is not inhibited by classical cytosolic GST inhibitor, in contrast to cPGES.

II.4.4. Features of mPGES-1 promoter

The human mPGES-1 promoter is GC rich and lacks a TATA box at a functional site and contains numerous potential transcription factor binding sites, including two GC-boxes, two tandem Barbie boxes and an AHR (aryl hydrocarbon response element). The putative promoter region of mPGES-1 was shown to be transcriptionally active and was inducted by IL-1β and down-regulated by Phenobarbital (Forsberg et al, 2000).

The mouse mPGES-1 promoter contains several transcription factor binding sites (Figure 5), including two tandem GC-boxes, C/EBPα and -β, AP-1, and three GRE and two progesterone receptors (PR). The tandem GC box sequences in the mPGES promoter play a major role in regulating its inducible transcription. Egr-1 (early growth response factor-1), an inducible zinc finger protein that recognizes the GC-rich consensus DNA sequence 5'-GCG(T/G) GGGCG-3' binds to the proximal GC box in the mPGES promoter region and facilitates inducible transcription of the mPGES gene. Egr-1 gene is rapidly and transiently induced by a variety of stimuli (TPA, cytokines, and LPS) or cellular stresses. Cytokine-induced mPGES-1 expression was regulated by Egr-1 (Naraba et al, 2002).

The sequence of the mouse and human mPGES promoters (-1 to –640) is only 48% homologous, but, the homology between them around the tandem GC boxes (-70 to –124) is relatively high 78%. Thus, the tandem GC boxes are critical for transcriptional activation of both the human and the mouse mPGES gene (Naraba et al, 2002).

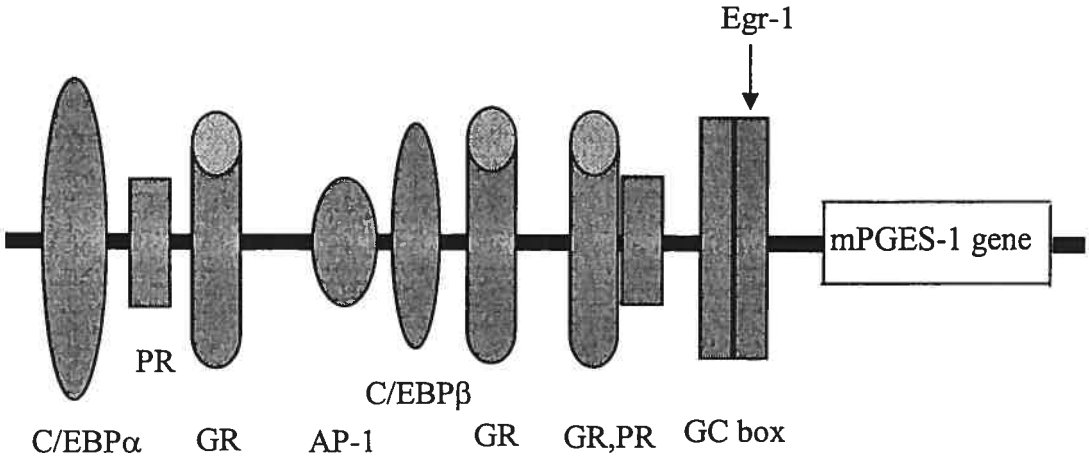


Figure 5. Regulatory elements in the mouse mPGES-1 promoter. Egr-1 binds to the proximal GC-box and triggers mPGES-1 transcription

II.4.5. Expression and regulation of mPGES-1

Tissue distribution of mPGES-1 in different species: The human tissue distribution was analyzed by Northern blot analysis. High expression of mPGES-1 mRNA was detected in A549 and HeLa cancer cell lines. Intermediate level of expression was demonstrated in placenta, prostate, testis, mammary gland, and bladder whereas low mRNA expression was observed in several other tissues (Jakobsson et al, 1999); Northern blotting of mPGES-1 in various mouse tissues revealed that the mPGES-1 mRNA was expressed intensely in the epididymis and weakly in the lung, spleen, skin, kidney, colon, and brain. No mRNA for mPGES-1 was detectable in the uterus, ovary, or oviduct of female mouse reproductive organs (Lazarus et al, 2002); A low basal expression of mPGES-1 had also been found in several rat tissues, but high constitutive expressions were seen in the stomach and in the thymus of rat (Mancini et al, 2001). The distribution of mPGES-1 mRNA expression was also studied in various bovine tissues by RT-PCR/Southern blot. Results showed that levels of mPGES transcripts varied across tissues. Levels of mPGES mRNA were highest in the seminal vesicle and in a preovulatory follicle obtained 24 h after HCG treatment, moderate to high in the stomach, intestine, pituitary and liver, and relatively low in other tissues tested (Filion et al, 2001). mPGES-1 may be involved in normal physiology.

The mPGES-1 was induced *in vitro* by various proinflammatory stimuli including LPS, IL-1β, TNF-α, β-amyloid, and phorbol 12-myristate 13-acetate (PMA). Various proinflammatory stimuli have been shown to co-ordinately induce mPGES-1 and COX-2 in several types of culture cells, often associated with increased PGE_2 production. Up-regulation of mPGES-1 was previously reported to occur in a human lung carcinoma-derived A549 cell line after treatment with IL-1β (Forsberg et al, 2000) and in human dermal fibroblasts and vascular smooth muscle cells after stimulation with IL-1β, TNF-α, PMA, and LPS (Soler et al, 2000). Up-regulation of mPGES-1 also occurred in human orbital fibroblasts after stimulation with IL-1β and TNF-α (Han et al, 2002) and in human rheumatoid synovial cells and OA articular chondrocytes after treatment with IL-1ß and TNF-α (Kojima et al, 2004). Moreover, the induction of mPGES-1 was demonstrated to take place in rat and mouse osteoblasts and peritoneal macrophages after stimulation with IL-1β, TNF-α, and LPS (Murakami et al, 2000), in

rat colon upon LPS stimulation (Mancini et al, 2001) and in rat astrocytes after stimulation with beta-amyloid (Satoh et al, 2000).

The induction of PGES has also been reported recently in two inflammatory models *in vivo*, including LPS-induced pyresis and adjuvant-induced arthritis (Mancini et al, 2001). Induction of PGES occured in tissues from Harlan Sprague-Dawley rats after LPS-induced pyresis *in vivo*. Rat PGES was up-regulated at the mRNA level in lung, colon, brain, heart, testis, spleen, and seminal vesicles. PGES and COX-2 were also up-regulated to the greatest extent in a rat model of adjuvant-induced arthritis. The RNA induction of PGES in lung and the adjuvant-treated paw correlated with a 3.8- and 16-fold induction of protein seen in these tissues by immunoblot analysis (Mancini et al, 2001). Furthermore, the regulation of PGES under physiological conditions had been described by using the bovine preovulatory follicle model, the results indicated that PGES expression was not constitutive in follicular cells but was induced by gonadotropins prior to follicular rupture (Filion et al, 2001).

The expression of mPGES is stimulus-inducible and is downregulated by anti-inflammatory glucocorticoids. mPGES-1 expression induced by cytokine was reversed by dexamethasone in A549 cells. The upregulation of mPGES-1 expression by IL-1β can be blocked by dexamethasone in human orbital fibroblasts (Han et al, 2002). Human synoviocytes from patients with RA express low levels of mPGES-1 and mPGES-1 mRNA and protein expression were significantly upregulated by IL-1β and dexamethasone inhibited mRNA and protein expression for mPGES-1 in IL-1β stimulated cells (Kojima et al, 2002). Dexamethasone markedly suppressed the LPS induced expression of mPGES in rat and mouse macrophages and osteoblasts (Murakami et al, 2000). Morever, COX-2-selective inhibitor NS-398, SC58125, rofecoxib, or meloxicam prevented IL-1β-induced mPGES-1 expression and this reduction of expression was reversed by PGE_2 (Kojima et al, 2003). PPAR-γ and PPARγ ligands are also involved in mPGES-1 regulation. PPARγ ligands have been shown to inhibit a number of inflammatory events. PPARγ ligands inhibited the expression of the inducible nitric oxide synthase, MMP-1, MMP-13, and COX-2 in human synovial fibroblasts and chondrocytes (Fahmi et al, 2002). Our previous study

showed that PPARγ ligands inhibited the expression of mPGES in human synovial fibroblasts (Cheng et al, 2004).

II.4.6. mPGES-1 implication in physiology and pathology

mPGES-1 is an inducible perinuclear enzyme that is functionally linked with COX-2 in marked preference to COX-1. COX-2 and mPGES-1 are essential components for delayed PGE_2 synthesis, which may be linked to inflammation, fever, osteogenesis, and even cancer. Recent reports demonstrated that mPGES-1 is an important enzyme in the production of PGE_2 related to the development of chronic inflammation in patients with arthritis.

Inflammation: The classical signs of acute inflammation are pain, swelling, fever, local reddening and loss of function. PGE_2 is a potent mediator of inflammation. Induction of mPGES following proinflammatory stimuli both in vitro and in vivo strongly suggested that this terminal enzyme is an essential component for PGE_2 production during the inflammatory response. mPGES-1 has been shown to be colocalized with COX-2 in mouse peritoneal macrophages stimulated with LPS (Murakami et al, 2000) and mPGES-1 is overexpressed in synovial tissues from patients with RA (Westman et al, 2004). Animal models of pyresis have been used to examine the role of mPGES-1 in fever. mPGES-1 and COX-2 co-expressed in the perinuclear region of brain endothelial cells of rats during LPS induced pyresis fever which was associated with increase in PGE_2 levels in the cebrospinal fluid (Yamagata & Matsumura, 2001). Mice with targeted mPGES-1 gene disruption do not produce PGE_2 and fail to develop fever in response to LPS (Engblom et al, 2003). These findings indicated that central PGE_2 synthesis by mPGES-1 is a general and critical mechanism for fever during infectious and inflammatory conditions. In addition, inflammatory pain hypersensitivity was reduced significantly in mPGES-1-deficient mice relative to WT mice, suggesting that mPGES-1 is involved in mediating acute pain during an inflammatory response (Kamei et al, 2004).

Arthritis: The role of mPGES-1 in arthritis was recently elucidated. The rat adjuvant-induced arthritis (AIA) model is one of the well-known animal model of human RA that has been used for the preclinical development of NSAIDs and COX-2 inhibitors. The expression of mPGES-1 has been examined in a rat model of AIA. Mancini et al

utilized this model to detect mPGE-1, and 5 days after adjuvant treatment, a significant increase in the inducible mPGE-1 was detected in treated paw and no mPGES-1 was detected in the naive (vehicle-treated) rat paw (Mancini et al, 2001). Similarly, in the same animal model, another report confirmed that the profile of induction of mPGES-1 (50- to 80-fold) in the adjuvant-treated paw was similar to that of COX-2 by both RNA and protein analysis. The induction of mPGES-1 was detected on day 1 and persisted to day 25 following adjuvant treatment in the primary paw. The maximum induction was measured at days 1–3 with a 60- to 80-fold increase of mPGES-1 as compared with the nonadjuvant-treated paw at time zero. These results show that mPGES-1 is up-regulated throughout the development of AIA and suggest that it plays a major role in the elevated production of PGE_2 in this model (Claveau et al, 2003).

The involvement of mPGES-1 in arthritis was also demonstrated in a collagen antibody-induced arthritis (CAIA), another model for human RA. In CAIA model, the arthritic symptoms were apparently mild in mPGES-1 KO mice compared with replicate WT mice. Moreover, bone destruction and juxtaarticular bone loss in CAIA were less obvious in mPGES-1 knockout mice than in replicate WT mice. Collectively, these results provide unequivocal evidence that mPGES-1 contributes to the formation of PGE_2 involved in pain hypersensitivity and inflammation (Kamei et al, 2004). Furthermore, induced expression of mPGES-1 was seen in human rheumatoid synovial cells and OA articular chondrocytes, after treatment with IL-1ß and TNF-α (Kojima et al, 2004). Intracellular mPGES-1 staining was observed in synovial membranes of RA patients studied. Specifically, strong expression of mPGES-1 was detected in synovial lining cells. The demonstration of mPGES-1 expression in synovial tissues from patients with RA suggests a role for mPGES-1 in the RA disease process (Westman et al, 2004). Taken together, it can be concluded that mPGES-1 is involved in various types of inflammation, including fever, pain hyperalgesia, and inflammatory arthritis.

Tumorigenesis: PGE_2 is one of the key prostanoids responsible for tumorigenesis. Both COX-2 and mPGES-1 are needed for efficient PGE_2 biosynthesis. Transfection of mPGES-1 in combination with COX-2 into HEK293 cells leads to cellular transformation, which is manifested by aggressive growth, pilling up and aberrant round-shape morphology (Murakami et al, 2000). In another study, co-expression of mPGES-1 and COX-2 resulted in colony formation in soft agar culture and tumor

formation when implanted into nude mice (Kamei et al, 2003). HCA-7, a human colorectal adenocarcinoma cell line that displays COX-2- and PGE_2-dependent proliferation, expressed both COX-2 and mPGES-1 constitutively. Treatment of HCA-7 cells with an mPGES-1 inhibitor or antisense oligonucleotide attenuated PGE_2 production and cell proliferation. These results suggest that aberrant expression of mPGES-1 in combination with COX-2 can contribute to tumorigenesis. Furthermore, the expression of mPGES-1 is markedly elevated in several types of cancer, such as in colon cancer, lung cancer and endometrial carcinoma.

Reproduction: Ovulation and fertilization are key processes in female reproduction, which is regulated by several hormones including gonadotropins. PGE_2, dominant prostanoid in the ovary, which play a central role in the inflammatory reaction, are also key mediators of ovulation. The predominant and obligatory role of PGE_2 is evidenced from the anovulatory phenotype observed in mice deficient for the PGE_2 receptor EP_2 (Tilley et al, 1999). In bovine female reproductive organs, mPGES-1 is highly expressed in granulosa cell layer of follicles after stimulation with gonadotropins. Induction of mPGES-1 by gonadotropins in the same cell type directly parallels the induction of COX-2. This study provided the first evidence of a marked up-regulation of mPGES-1 in the hours just prior to ovulation, a process during which PGE_2 synthesis is obligatory (Filion et al, 2001). In monkey granulosa cells, expression of mPGES-1 mRNA and protein by granulosa cells of periovulatory follicles increased in response to HCG (human chorionic gonadotrophin) administration, peaking just before the expected time of ovulation. Monkey granulosa cells also expressed mPGES-2 and cPGES mRNA, but mRNA levels did not change in response to HCG administration. These data suggest that mPGES-1, a gonadotropin up-regulated PG synthesis enzyme, may be the primary PGES responsible for the increased follicular PGE_2 levels necessary for primate ovulation (Duffy et al, 2005). mPGES-1 is also constitutively expressed in male reproductive organs at high levels. In male mice reproductive tract, the expression of mPGES-1 increased from the testis to the cauda epididymis and was highest in the vas deferens (Lazarus et al, 2002).

Bone metabolism: Bone remodelling, comprising resorption of existing bone and de novo bone formation, is required for the maintenance of a constant bone mass. PGs, particularly PGE_2, are produced by bone and have potent stimulatory effects on bone

resorption and bone formation. They stimulate the differentiation of precursors of both the bone osteoclasts and the bone osteoblasts, they stimulate bone resorption in vitro, and stimulate bone formation when PGs are administered exogenously in vivo (Pilbeam et al, 1996). The production of PGE_2 in bone is highly regulated by several proinflammatory cytokines. The multifunctional regulation is probably mediated by different PG receptors. Bone resorption caused by proinflammatory stimuli in vivo is impaired in mice deficient in PGE_2 receptor EP_4 (Sakuma et al, 2000). In cultured rat and mouse osteoblastic cells, proinflammatory cytokines induce mPGES-1 expression, which occurs in parallel with the induction of COX-2 and the generation of PGE_2 (Murakami et al, 2000). In the mouse coculture system of osteoblasts and bone marrow, an antisense oligonucleotide blocking mPGES expression inhibited not only PGE_2 production, but also osteoclastogenesis and bone resorption stimulated by the cytokines, which was reversed by addition of exogenous PGE_2 (Saegusa et al, 2003). These observations suggest that mPGES-1, acting downstream of COX-2 is involved in bone metabolism.

II.4.7. cPGES

cPGES is a 23 kDa cytosolic protein that is identical to p23, a heat shock protein 90 (Hsp90)-binding protein, which has been originally implicated as a cofactor for the molecular chaperone function of Hsp90. cPGES is highly conserved among animal species (>95%). The murine cPGES gene spans approximately 22 kb and consists of eight exons. The cPGES gene promoter is GC-rich and contains many SP1 sites but lacks an obvious TATA box motif (Zhang et al, 2003). cPGES enzymes belong to the GST superfamily and require GSH as an essential co-factor for its activity. The homology between cPGES and other cytosolic GSTs is low (20%), but they all share a conserved tyrosine near the N-terminus (Tyr9), which is known to be critical for the activity of cytosolic GSH S-transferase, was essential for PGES activity (Tanioka et al, 2000).

The expression of cPGES/p23 is constitutive and is unaltered by proinflammatory stimuli in various cells and tissues, except that it is increased significantly in rat brain after LPS treatment. cPGES is mainly localized to the

cytosol, but can move to the endoplasmatic reticulum after Ca^{2+} -ionophore challenge. Cotransfection and antisense experiments suggest that cPGES is functionally coupled to COX-1 for the generation of PGE_2 particularly during the immediate PGE_2 biosynthetic response to increasing Ca^{2+}. Thus, functional coupling between COX-1 and cPGES/p23 may contribute to production of the PGE_2 that plays a role in maintenance of tissue homeostasis (Tanioka et al, 2000).

II.4.8. mPGES-2

A novel type of mPGES (termed mPGES-2), which was originally purified from the microsomal fraction of bovine heart (Watanabe et al, 1999), has been cloned. The gene for human mPGES-2 maps to chromosome 9q33-34. The cDNA encodes a 41 kDa protein that contains an N-terminal hydrophobic region and the consensus region of glutaredoxin and of thioredoxin. The mPGES-2 enzyme is activated by various SH-reducing reagents and is non-specific for GSH. Moreover, the mPGES-2 mRNA distribution was high in the heart and brain, but not expressed in the seminal vesicles. In several cell lines, mPGES-2 promoted PGE_2 production via both COX-1 and COX-2 in the immediate and delayed responses with modest COX-2 preference. In contrast to the marked inducibility of mPGES-1, mPGES-2 was constitutively expressed in various cells and tissues and was not increased appreciably during tissue inflammation or damage (Murakami et al, 2003). Collectively, mPGES-2 is a unique PGES that can be coupled with both COXs and may play a role in the production of the PGE_2 involved in both tissue homeostasis and disease.

III. PPARγ and 15d-PGJ_2

III.1. Peroxisome proliferator-activated receptors (PPARs):

PPARs are transcriptional factors belonging to the ligand-activated nuclear hormone receptor super-family, which includes the steroid, retinoid, and thyroid hormone receptors. To date, three major type of PPAR, encoded by separate genes, have been identified; they are PPARα (NR1C1), PPARδ/β (NR1C2), and PPARγ (NR1C3). The PPARs display different tissue distributions and appear to serve different biological functions. Natural fatty acids and the fibrate class of hypolipidaemic drugs are known activators of PPARs (Wahli et al, 1995). The actions of PPAR were originally thought to be crucial for controlling lipid and glucose metabolism. Recent studies have shown that PPAR also regulates inflammatory responses, immune response, cell growth, differentiation, proliferation and apoptosis (Escher & Wahli, 2000; Corton et al, 2000). In addition, PPAR is also involved in the regulation of various types of tumours, inflammation, atherosclerosis, obesity and diabetes.

The nuclear receptors have a highly conserved modular structure organized into functional domains. Similar to other nuclear receptors, all three PPAR isoforms contain four major functional domains called A/B, C, D and E/F. The N-terminal A/B domain contains a ligand-independent activation function 1 (AF-1) responsible for the phosphorylation of PPAR. The DNA binding domain or C domain promotes the binding of PPAR to the peroxisome proliferator response element (PPRE) in the promoter region of target gene. The D site is a docking domain for cofactors. C-terminal E/F domain include ligand-binding domain responsible for ligand specificity and activation of PPAR binding to the PPRE, which increases the expression of targeted genes and the ligand-dependent transactivation domain (AF-2 domain). Gene mapping studies demonstrated that these three PPAR isoforms are encoded by distinct genes mapping to human chromosome 22, 6, and 3, respectively (Desvergene & Wahli, 1999).

The gene transcription mechanism is identical in all PPAR subtypes. The process of transcription begins with the binding of ligands (endogenous or exogenous) to the PPAR receptor. Ligand-bound PPAR heterodimerises with retinoid X receptors (RXR), a subfamily of molecules within the nuclear receptor superfamily that are

activated by 9-cis retinoic acid, this heterodimer bind to specific PPRE, located in the promoter regions of target genes (Ijpenberg et al, 1997). PPRE consists of a direct repeat of hexameric core recognaition elements spaced by 1bp (DR1, 5'AGGTCANAGGTCA-3'). After activation of the PPAR/RXR heterodimer at the PPRE, the PPAR/RXR complex can recruit diverse nuclear receptor co-factors (coactivators and corepressors) that modulate transcriptional activity of PPAR and RXR receptor heterodimer. This results in the expression and/or repression of a variety of genes whose promoters contain PPRE. In addition to RXR, a number of PPAR interactive proteins (cofactors) have been shown to associate with PPAR such as p300/CBP, the steroid receptor coactivator-1, c-jun p65, and nuclear factor of activated T cells (NF-AT). The expression level of PPAR receptors, the chemical properties and local concentrations of PPAR-specific ligands, and the availability of these co-factors all contribute to the biologic effect of PPAR activation or inactivation.

Three PPARs are differentially expressed among tissues. PPARα is highly expressed in tissues that possess high mitochondrial and β-oxidation activity, including liver, renal cortex, intestine mucosa, and heart. PPARγ is highly enriched in adipose tissue, but lower expression in urinary bladder, intestine, kidney, heart, liver, and vasculature. PPARβ/δ seem to be ubiquitously expressed at low levels in many tissue (Mukherjee et al, 1994). In addition, PPAR is present in the joint connective tissue cells including chondrocytes, synoviocytes and osteoclasts.

III.2. PPAR ligand

The ligand-binding domains of the three PPAR isotypes have sufficiently divergent amino acid sequences to allow some ligand specificity. PPAR ligands are classified in synthetic ligands, such as hypolipidemic, anti-inflammatory and insulin-sensitizing compounds and in natural ligands, such as medium and long-chain fatty acids and eicosanoids. Although many fatty acids are capable of activating all three PPAR isoforms, some preference for specific fatty acids by each PPAR has been demonstrated. Synthetic ligand, the hypolipidemic agent WY-14643 is potent ligand for activating PPARα. This compound does not bind to or activate PPARγ or β and therefore serves as a useful pharmacological tool to selectively modulate PPARα

activity. Endogenous AA COX metabolite prostacyclin, the linoleic acid 15-lipoxygenase-1 product 13-S-hydroxyoctadecadienoic acid, and synthetic compounds including L-165041 and GW2433 have been found to be selective PPARβ/δ ligands. (Shureiqi et al, 2003).

Natural PPARγ ligands include: polyunsaturated fatty acids such as linoleic acid, AA and eicosapentaenoic acids; oxidized lipids such as 9-hydroxy-octadecadienoic acid (9-HODE), 13 HODE and 15-hydroxyeicosatetraenoic acid (15-HETE); and PGD_2 metabolite 15d-PGJ_2 which was the first endogenous PPARγ ligand identified. This prostaglandin is currently widely utilized as a naturally occurring PPARγ activator. Synthetic PPARγ ligands antidiabetic thiazolidinediones (TZD) including troglitazone, ciglitazone, pioglitazone and rosiglitazone are potent PPARγ selective agonists and very effective in improving glycemic control via insulin sensitization (Lehmann et al, 1995). Two of these drugs, rosiglitazone and pioglitazone, are in widespread clinical use for the treatment of type 2 diabetes. In addition to TZD, other synthetic compounds have been identified as PPARγ agonists. Several NSAID, such as indomethacin, ibuprofen, fenoprofen, and flufenamic acid, bind and activate PPARγ and promote adipocyte differentiation (Lehmann et al, 1997).

III.3. 15d-PGJ_2

III.3.1. 15d-PGJ_2 synthesis

The cyclopentenone prostaglandins PGJ series contain a cyclopentenone ring structure, which is characterized by the presence of a chemically reactive α, β-unsaturated carboyl. The members of the cyclopentenone prostaglandin family have anti-infalmmatory, anti-neoplastic, anti-viral activities. In contrast with the PGs, which elicit a biological response by binding to G-protein coupled receptors, cyclopentenone prostaglandin PGJ_2 interacts with other specific cellular targets, including signalling molecules and transcription factors.

Synthesis of 15d-PGJ_2 begins with the sequential action of three classes of enzymes. AA generation by PLA_2 initially regulates synthesis of all PGs. AA then can be oxidized by COXs, yielding PGH_2, which is in turn, sequentially converted to various PGs. PGD_2 is generated by the action of hematopoietic and /or lipocaline PGD_2

synthases (H-PGDS, L-PGDS). PGD_2 undergoes chemical dehydration, losing water to form the cyclopentenone prostaglandin PGJ_2. The final product of this pathway, PGJ_2 is then nonenzymatically converted into15d-PGJ_2. However, no specific 15d-PGJ_2 synthase has been identified. Rather, 15d-PGJ_2 is a derivative of PGD_2, and its synthesis initially depends upon the enzymatic machinery for PGD_2 generation.

15d-PGJ_2 was initially identified as a product of albumin-catalyzed transformation of PGD_2 in vitro (Fitzpatrick & Wynalda, 1983). To establish a physiological role for 15d-PGJ_2 or PGJ series, one needs to demonstrate that they exist in vivo in sufficient quantities to elicit a biological response. There are some evidences that the PGJ series do exist in vivo. For example, previous studies showed that delta 12-PGJ_2, a downstream metabolite of PGD_2 is present in significant quantities in human urine. It is formed naturally in the body and excreted as a urinary PGD_2 metabolite. In 1999, Gilroy et al. identified 15d-PGJ_2 in inflammatory fluids by using an EIA method and demonstrated that levels of this compound increase during the resolution phase of inflammation. Recent study by Shan et al demonstrated the presence of 15d-PGJ_2 in arthritic synovial fluids, though at picomolar levels, using a recently- developed ELISA assay (Shan et al, 2004). Much excitement was generated in 1995 when this compound was found to be a high affinity ligand for the PPARγ. It is crucial to note that PGD_2 is among the most abundant prostaglandins in synovial fluid (Pietila et al, 1984). Therefore, it is likely that PGD_2 derivatives 15d-PGJ_2 are present in sufficient amounts to activate PPARγ and act as negative feedback loop for inflammation in vivo.

III.3.2. PGD synthase and PGD2

PGD_2 is naturally and rapidly converted into PGJ_2 by noenzymatic pathways, and PGJ_2 itself is rapidly metabolized to Δ^{12} –PGJ2 and 15d-PGJ_2 after elimination of one or two water molecules. Therefore, PGD synthase could be as PGJ_2-synthesizing enzyme. PGD_2 is formed abundantly in several tissues, most notably in mast cells and in the brain. PGD synthase catalyzes the isomerization of PGH to PGD, which acts as an endogeous somnogen and an allergic mediator. There are two distinct types of PGDS in mammals: One of them is lipocalin-type PGDS (L-PGDS) localized in the central nervous system, male genitals, and heart; and the other is hematopoietic PGDS

(H-PGDS) in mast cells, antigen-presenting cells and Th lymphocytes. L-PGDS is the same as beta-trace, a major protein in human cerebrospinal fluid, and is also secreted into the seminal plasma and plasma. The L-PGDS concentration in various body fluids is useful as a marker for various diseases such as renal failure and coronary atherosclerosis. H-PGDS, a cytosolic enzyme, is a member of the Sigma class of GST. It is H-PGDS that controls PGD_2 production in various peripheral tissues. Recent studies reveled that human articular chondrocytes constitutively express L-PGDS and that proinflammatory cytokines up-regulate H-PGDS mRNA expression in these cells (Shan et al, 2004).

III.3.3. PPARγ-dependent 15d-PGJ_2 actions

Prostaglandins of the J_2 series form in vivo and exert effects on a variety of biological processes. While most of PGs mediate their effects through G protein-coupled receptors, the mechanism of action for the J_2 series of PGs remains unclear. In 1995, 15d-PGJ_2 was identified as both a PPARγ ligand and an inducer of adipogenesis. Thus, adipogenic prostanoids initiate key transcriptional events through a common nuclear receptor signaling pathway and suggest a novel mechanism of action for PGs of the J_2 series (Forman et al, 1995). Although the affinity of 15d-PGJ_2 for PPARγ is significantly lower then that of classical steroid hormones for their cognate intracellular receptors, it represents the highest affinity natural ligand for PPARγ identified to date. The binding of ligand 15d-PGJ_2 to PPARγ results in the expression and/or repression of a variety of genes whose promoters contain PPRE. The LPS induced transcription responses of AP-1, NF-κB and STAT1 can be repressed by 15d-PGJ_2 in PPARγ dependent manner (Ricote et al, 1998). 15d-PGJ_2 represses the COX-2 transcription gene via PPARγ, thereby exerting a negative feedback control on its own biosynthesis. On the other hand, 15d-PGJ_2 has been shown to enhance gene expression such as TNF-α, IL-6 and IL-8. The mechanism of transcriptional activation involves the recruitment of coactivator complexes to the promoter. Alternatively, the receptor-dependent transcriptional repression of genes, involves negative regulation of the activity of other transcription factors such as AP-1 and NF-κB.

III.3.4. PPARγ-independent 15d-PGJ_2 action

There are numerous reports demonstrating PPARγ dependent mechanisms of action of 15d-PGJ_2, but there are also experiments showing a PPARγ-independent action of 15d-PGJ_2. For instance, 15d-PGJ_2 repressed iNOS promoter activity in cells that did not express PPARγ (Petrova et al, 1999). Comparison of the protency of different PPARγ ligands in inhibiting inflammatory gene expression in macrophages showed that relatively high concentrations of TZD, which are considered highly selective for PPARγ, were needed to promote effects similar to those of the less specific PPARγ ligand 15d-PGJ_2, suggesting that PPARγ independent mechanisms might be involved in these processes. Therefore, 15d-PGJ_2 can have PPARγ-independent actions. At least two identified candidates can reasonably be suspected to mediate PPAR-independent actions: the PG receptors and the NF-κB system.

PG receptors: Unlike other PGs, 15d-PGJ_2 has no known specific membrane receptor, even though PGJ_2 can signal through PGD_2 receptor with the same potency as PGD_2 itself. Two PGD_2 receptors have been identified: DP_1 receptor and DP_2 receptor (also designated $CRTH_2$, chemoattractant receptor-homologous molecule expressed on T helper (Th)2 cells). $CRTH_2$ differs from DP in its signal pathways: $CRTH_2$ is coupled with Gi-type G protein and DP is coupled with Gs-type G protein. PGD_2 acts via the DP_1 receptor and Gs to activate adenyl cyclase and raise intracellular cAMP levels and PKA activity. Several studies suggested that 15d-PGJ_2 has a weak ligand affinity on DP_1 receptors in human neutrophils, and in a human natural killer cell line (Zhang & Young, 2002). PGD_2 acts through the $CRTH_2$ receptor and Gαi and plays important roles in allergic inflammation, through induction of chemotactic migration and /or activation of Th2 cells, eosinophils, and basophils (Tanaka et al, 2004). Resent studies indicated that 15d-PGJ_2 activates $CRTH_2$ on eosinophils, increasing intracellular calcium fluxes with a potency nearly equal to that of PGD_2, the principal ligand for the receptor (Monneret et al, 2003). Thus, the DP receptors have a role in mediating some of the PPARγ-independent effects of 15d-PGJ_2.

Nuclear factor-κB (NF-κB) system: There is abundant evidence implicating NF-κB as a major target for receptor-independent gene repression by 15d-PGJ_2. NF-κB is a transcription factor, which resides in the cytoplasm in association with its repressor I-

κB. In response to signaling by inflammatory cytokines, I-κB can be phosphorylated by the I-κB kinase (IKK), resulting in the release of NF-κB and its migration to the nucleus and activation of gene expression. 15d-PGJ_2 inhibits NF-κB either by inhibiting the I-κB kinase, thereby preventing I-κB degradation and nuclear entry of NF-κB or by directly interacting with NF-κB to inhibit binding of NF-κB to target DNA sequences. One study demonstrated that 15d-PGJ_2 repressed the activity of an NF-κB reporter construct. Repression of this reporter occurred in the absence of PPARγ, although the effect was enhanced in the presence of 15d-PGJ_2 (Ricote et al, 1998). This clearly indicated that NF-κB was repressed by 15d -PGJ_2 via PPARγ-dependent as well as PPARγ-independent mechanism.

Furthermore, COX-2 is under the control of NF-κB and its expression is inhibited by 15d-PGJ_2. COX-2 is negatively regulated by 15d-PGJ_2 via at least three routes, one involving PPARγ-mediated repression of NF-κB and the others resulting from a direct action of PGs on the I-κB/ NF-κB system. Direct inhibition of NF-kB signalling by 15d-PGJ_2 may contribute to negative regulation of prostaglandin biosynthesis and inflammation (Straus et al, 2000).

It is now known that 15d-PGJ_2 is both an endogenous PPARγ ligand as well as a direct inhibitor of several other signal transduction pathways. The consequences of these activities are complex, but are very likely to play a role in the prevention and/or resolution of inflammation.

III.4. PPARγ

To date, three splice variants of the PPARγ isoform, designated PPARγ1, PPARγ2, and PPARγ3 have been identified. PPAR expression is tissue dependent. PPARγ1 is found in a broad range of tissues, whereas PPARγ2 is restricted to adipose tissues. PPARγ3 is abundant in macrophages, the large intestine and white adipose tissue (Braissant et al, 1996).

IV. Proinflammatory cytokines

The term cytokine, or immunocytokines, was used initially to separate a group of immunomodulatory proteins, called also immunotransmitters, from other growth factors that modulate the proliferation and bioactivities of non-immune cells. Today the term cytokine is used as a generic name for a diverse group of soluble proteins and peptides, which act as humoral regulators at nano- to picomolar concentrations and which, either under normal or pathological conditions, modulate the functional activities of individual cells and tissues. Cytokines are soluble or cell surface molecules that play an essential role in mediating cell-cell interactions and regulate processes taking place in the extracellular environment. Within tissues, cytokines exert their effects through autocrine, paracrine, and juxtacrine action. Many growth factors and cytokines act as cellular survival factors by preventing programmed cell death. The cloning of cytokines through recombinant DNA technologies established the full spectrum of the functional properties of the individual cytokines and pleiotropy of their biologic activities.

Cytokines rarely exert their biological activities in isolation and most often they act in concert with multiple other cytokines that may or may not be members of the same family. The interaction of these structurally and functionally distinct factors in highly ordered temporal and spatial sequence creates a cytokine network that ultimately determines the response pattern within a given tissue. The integration of the activities of the individual cytokines within the cytokine network provides a mechanism for additive or synergistic interactions and system for regulating biological processes through a balance of inhibitory and stimulatory effects. In addition, the networks provide a system for feedback regulation. The effects of cytokines on cells may be modulated by the induction of products that can act back on target cells to modulate their function by autocrine or paracrine mechanisms.

It is believed that cytokines play an important role in the pathophysiology of OA. They are closely associated with functional alterations in synovium, cartilage and subchondral bone, and are produced both spontaneously and following stimulation by the joint tissue cells. They appear to be first produced by the synovial membrane and diffused into the cartilage through the synovial fluid. They activate the chondrocytes,

which in turn could produce catabolic factors such as proteases and proinflammatory cytokines through auto- and parcrine mechanisms. In OA synovium, the synovial lining cells are key inflammatory effectors.

The major cytokines believed to be involved in cartilage metabolism in OA include: catabolic cytokines IL-1α, IL-1β, TNF-α, IL-17, IL-18; oncostatin M; regulatory cytokines IL-6 and IL-8; as well as inhibitory cytokines IL-4, IL-10, IL-13 and IL-1 receptor antagonist (IL-1Ra). Several anabolic cytokines (growth factors) also appear to be involved in this disease, such as TGF-β, FGF, and insulin-like growth factor (IGF).

IV.1. IL-1β

Monocytes are the main source of secreted IL-1. They express predominantly IL1-β while human keratinocytes express large amounts of IL-1α. Murine macrophages display a transition from IL-1β to IL-1α production during maturation of monocytes into inflammatory macrophages.

IL-1β is synthesized as precursors of approximately 35 kDa (269 amino acids). The mature proteins are generated by proteolytic cleavage by a number of proteases. Active form of IL1-β is 17 kDa, 153 amino acids. IL1-1β encoded gene has length of 9.7 kb and comprise seven exons. Its mRNA has a length of 1.6-1.7 kb. The human IL1-β gene maps to chromosome 2q13-q21. In articular joint tissue, including synovial membrane, synovial fluid and cartilage, IL-1β has been found in the active form.

The biological activation of IL-1 in cells is mediated through association with specific cell-surface receptors (IL-R). Two receptors have been identified, type I and type II, with type I being responsible for signal transduction. IL-1β binds to the membrane-bound IL-1 type I receptor (IL-1RI), leading to the recruitment of the IL-1 receptor accessory protein (IL-1RacP). This heterotrimeric complex transduces a signal to the cell nucleus, culminating in production of inflammatory and destructive mediators. The IL1-β but not the IL1-α precursor must be processed before it can bind to the receptor. Both type of IL-1R can also be shed from the cell surface, and they are named IL-1 soluble receptors (IL-1sR). For IL-1, the shed receptor may function as a receptor antagonist because the ligand-binding region is preserved, and thus is capable

of competing with the membrane-associated receptors of the target cells. It has been shown that the elevated expression of type I IL-R in chondrocytes and synovial fibroblasts in OA may render these cells more sensitive to stimulation by IL-1 (Martel-Pelletier et al, 1992), thereby increasing their potential to secrete MMP and mediate joint destruction.

IL-1 receptor mediated signal transduction involves adenylate cyclase which transiently increases intracellular cAMP levels. A cAMP-dependent protein kinase (PKA) and a pertussis toxin-sensitive GTP binding protein of 46 kDa are also involved. Binding of IL-1 to its receptor activates transcription factor NF-kB.

The action of IL-1 can be inhibited by IL-1Ra, a natural competitive inhibitor of IL-1. IL-1Ra has a high affinity for the IL-1RI. However, binding of the inhibitory protein to the receptor does not allow the recruitment of the IL-1RacP, thus there is no signal transduction. The strong binding of IL-Ra to IL-1RI blocks the access of IL-1α and IL-1β to the receptor. It is believed that IL-1Ra has a pivotal role in homeostasis in the joint. Its production in OA synovium may not be in sufficient amounts to inhibit the effects of locally producted IL-1.

Role of IL-1β in cartilage degradation: IL-1β is the prototypical proinflammatory cytokine implicated in the pathogenesis of cartilage matrix degradation in OA. In the early studies, IL-1 soluble factor, originally termed catabolin, produced by normal, noninflamed porcine synovial fragment cultures was shown to stimulate chondrocytes to break down the surrounding cartilage matrix (Dingle et al, 1979). Similar activities in culture supernatants from mononuclear cells and synovium were found to activate secretion of prostaglandins and to stimulate the production of proteolytic enzymes by chondrocytes (Dayer et al, 1984). Subsequently, the catabolin isoforms were later identified as IL-1α and IL-1β with the capacity to induce chondrocyte-mediated cartilage degradation (Saklatvala et al, 1984).

IL-1β is considered to be one of the most potent catabolic factors in joint disease. First, it is produced in considerable quantities in OA joints, among other cells, by OA chondrocytes. IL-1β and other proinflammatory cytokines are present in OA synovial fluids and are expressed in the synovial tissues of patients with early OA. Immunohistochemical studies of cartilage showed the presence of IL-1β in OA

chondrocytes, predominantly in the superficial layer. Second, IL-1β is the most powerful driving force for the production of destructive proteases. IL-1β can induce joint articular cells, such as chondrocytes and synovial cells to produce other cytokines such as IL-8, IL-6, Leukemia inhibitory factor and their own production, as well as stimulate proteases and PGE_2 production. Third, it was also shown that IL-1β could contribute to the depletion of the cartilage matrix by decreasing the synthesis of cartilage-specific collagens and proteoglycans. Finally, IL-1β also potently involves induction of the expression of MMPs and other inflammatory factors, including iNOS, COX-2, mPGES-1 and PLA_2 which can modulate chondrocyte function in OA. Chondrocytes are capable of expressing these enzymes when stimulated by IL-1 alone or in combination with TNF-α. These all support a role for IL-1β in the pathogenesis of cartilage matrix breakdown in OA. Studies in animal models in vivo provide further evidence implicating a role for IL-1β in cartilage degradation in OA. For example, intra-articular injection of highly purified or recombinant IL-1β into the rabbit induced depletion of cartilage proteoglycan (Pettipher et al, 1986). Moreover, IL-1β-deficient mice were found to have normal inflammatory responses, but defective acute-phase responses, resistance to type II collagen-induced arthritis, and protection against cartilage destruction in streptococal cell wall-induced arthritis (Zheng et al, 1995).

IV.2. TNF-α

In mammals, TNF-α is secreted by macrophages, monocytes, neutrophils, T-cells, NK-cells following their stimulation by bacterial LPS. Stimulated peripheral neutrophilic granulocytes and also a number of transformed cell lines, astrocytes, microglial, smooth muscle cells, and fibroblasts also secrete TNF-α.

Human TNF-α is a non-glycosylated protein of 17 kDa and a length of 157 amino acids. Murine TNF-α is N-glycosylated. TNF-α forms dimers and trimers.The great majority of the cellular actions for TNF-α correspond to the secreted, soluble form of mature TNF-α. The 17 kDa mature form of TNF-α is produced by processing of a precursor protein of 233 amino acids. During its synthetic process, the mature form of TNF-α is formed by proteolytic cleavage of the extracellular portion of 26 kDa transmembrane pro-TNFα. A TNF-α converting enzyme (TACE) has been shown to

mediate this conversion. Therefore, TNF-α is expressed in two different forms: a soluble, mature 17 kDa form and a transmembrane 26 kDa form. The TNF-α gene has a length of approximately 3.6 kb and contains four exons. The primary transcript has a length of 2762 nucleotides and encodes a precursor protein of 233 amino acids. The amino terminal 78 amino acids function as a presequence. The human gene maps to chromosome 6p23-6q12.

TNF-α also acts by binding to two specific receptors on the cell membrane, named according to their molecular weight: TNFR1 (p55) and TNFR2 (p75). These two TNF-α receptors are transmbrane glycoproteins with a high degree of structural homology and expressed virtually in all cell types. It is known that TNFR1 and TNFR2 both bind TNF-α with high affinity. Althrough it has been generally believed that TNFR1 mediates the cellular actions of TNF-α, recent evidence points towards an important role for TNFR2 in mediating, at least in part, the biological activities of TNF-α. Both type receptors appear to be actively involved in signal transduction and both receptors are linked to distinct intracellular second-messengers. As is the case for IL-1R, both TNFR1 and TNFR2 can also be shed from cell surface. Similar to the TNF-α ligand, membrane-bound TNFR1 and TNFR2 can be proteolytically cleaved to release soluble forms of the receptors, which are able to bind TNF-α and, as a result, block the TNF activity or stabilize the trimeric conformation to maintain activity. In articular tissue cells, TNFR1 seems to be the dominant receptor responsible for mediating TNF-α activity. In OA chondrocytes and synovial fibroblasts, there is enhanced expression of TNFR1.

Role of TNF-α and synergism with IL-1: In OA, TNF-α also appears to be an important mediator of matrix degradation and a pivotal cytokine in synovial membrane inflammation, althrough this cytokine is detected in OA articular tissue at a low level. Studies both in vitro and in vivo showed that the effects of TNF-α were similar to or synergistic with IL-1 (α and β) and also suggested a role for this cytokine in cartilage destruction. Chondrocytes are the most likely source of TNF-α in the OA joint, because OA chondrocytes show high expression of this cytokine compared with OA synovial cells (Melchiorri et al, 1998). OA cartilage displays elevated messenger RNA levels of TNF-α and the TNF convertase enzyme as compared with those of normal cartilage

(Amin, 1999). The increase in TNF convertase enzyme will result in enhanced production of functional TNF-α. TNF-α shows effects on chondrocytes that are similar to those of IL-1, including stimulation of the production of matrix-degrading proteinases and suppression of cartilage matrix synthesis. This cytokine also induces chondrocytes to synthesize imflammatory factors, such as PGE_2 and NO. TNF-α is considered to be far less potent than IL-1β as a destructive mediator, but it is probably an important driving force of IL-1β synthesis. Synovial fibroblasts from OA joints show enhanced TNF-α receptor expression, and focal loss of articular cartilage in OA joints is associated with upregulation of the TNF-α p55 receptor (Webb et al, 1997). In animal models of RA, treatment with TNF-binding proteins such as the soluble TNF receptor, or the IL-1 receptor antagonist revealed that the presence of TNF-α was sufficient to drive inflammation at the onset of arthritis, while IL-1β had a pivotal role in sustaining both the inflammation and cartilage erosion (van den Berg et al, 1999). It can be concluded that TNF-α is a pivotal mediator of inflammation, whereas IL-1β is the most potent mediator of cartilage destruction.

IL-1β is much more potent than TNF-α. However, the activities of these two cytokines produce strong synergistic effects. Animal studies showed that intraarticular injection of recombinant preparations of IL-1 (α and β) stimulated the destruction of the articular cartilage, while injection of recombinant or purified preparations containing both TNF-α and IL-1 (α and β) elicited more severe cartilage damage than did injection of either cytokine alone (Page-Thomas et al, 1991). Clinical studies of combination therapy are in progress to assess safety and efficacy in patients with RA. Further work is required to determine whether cytokine synergism extends to OA.

IV.3. IL-17

IL-17 is proinflammatory cytokine secreted by activated T lymphocytes, predominantly of the CD4+ subtyte. It encodes a glycoprotein of 155 amino acids sequence. 20-30 kDa of this cytokine presents as a homodimer with variable glycosylated polypeptides.

The IL-17 protein family binds to a unique mouse receptor. A cDNA encoding a human homologue (CDw217) of the murine IL-17 receptor isolated from a human T-

cell library is 69% identical with the murine receptor and shares no homology with previously identified cytokine receptor families. Expression of the human receptor gene displays a broad tissue distribution. The human gene was localized to chromosome 22. Recombinant human IL-17 binds to the human receptor with low affinity.

IL-17 enhances expression of the intracellular adhesion molecule-1 (ICAM-1) in human fibroblasts. Human IL-17 also stimulates epithelial, endothelial, or fibroblastic cells to secrete IL-6, IL-8, and PGE_2. In the presence of human IL-17, fibroblasts can sustain the proliferation of CD34 (+) hematopoietic progenitors and their preferential maturation into neutrophils.

IL-17 is additional cytokine that is a potent inducer of catabolic responses in chondrocytes. It presents in RA synovial fluid and tissue. This cytokine increases the expression of IL-1β, IL-6, iNOS and COX-2 in human articular chondrocytes, and stimulates the production of IL-6, NO, COX-2 and MMPs. IL-17 also increases degradation and suppresses the synthesis of cartilage proteoglycans independent of IL-1β. Adenoviral over-expression of IL-17 induces cartilage proteolycan loss in the joint with minor cartilage erosion and enhances the development of cartilage erosions induced by collagen-induced arthritis (Lubberts et al, 2001). However, whether this cytokine has roles in the pathogenesis of OA has not been well established.

Recently, numerous studies have described synergistic or additive effects between IL-1β, TNF-α, and IL-17 in many systems in vitro and in vivo. For example, in human OA synovial fibroblasts, the combination of IL-17 and TNF-α synergistically stimulated production of IL-1β, IL-6, and IL-8, and IL-1β and TNF-α synergize to induce PGE_2 production and COX-2 expression in rabbit articular chondrocytes (Berenbaum et al, 1996). Furthermore, in vivo studies confirmed the above results. On human RA bone explants, the combination of IL-17 and IL-1β had a much larger effect on IL-6 production than either cytokine alone. IL-1β or IL-17 increased bone resorption and decreased formation and combination of IL-1β and IL-17 in these conditions increased the effect. Synergy between IL-1β, TNF-α and IL-17 on NO and PGE_2 production by explants of human OA knee menisci was also demonstrated (LeGrand et al, 2001).

In vitro and in vivo studies show that cytokines, acting individually or synergistically in networks, can profoundly alter chondrocyte activity. The role of cytokines in the pathogenesis of cartilage destruction in RA and related form of inflammatory arthritis is well established. Several lines of evidence suggest that cytokines may also play a role in abnormal chondrocyte function and cartilage matrix destruction in OA. The recent demonstration of clinical improvement in a small number of patients with OA treated with intraarticular IL-1Ra indicates the need for further investigation of the role of cytokines in the pathogenesis of dysregulated chondrocyte function in OA.

V. Research hypothesis

PGE_2 plays a critical role in inflammation and the pathophysiology of articular joint diseases, such as RA and OA. Recently, an inducible mPGES-1 was identified. This enzyme is functionally coupled with COX-2 and converts the COX product PGH_2 to PGE_2. Pro-inflammatory cytokines, IL-1β and TNFα, have been shown to induce mPGES-1 expression in several tissues and cell types, including synovial fibroblasts and osteoblasts. However, little is known about the expression and regulation of mPGES-1 in cartilage. We hypothesize that mPGES-1 plays a role as a modulator of inflammation in the regulation of PGE_2 production in joint tissues of OA patients. We analyzed mPGES-1 expression in normal and OA cartilage and explored the effects of different inflammatory agonists on the expression of mPGES-1 in OA chondrocytes.

B. ARTICLE

The Journal of Rheumatology 2005,32(5):887-95

Expression and Regulation of Microsomal Prostaglandin E Synthase-1 in Human Osteoarthritic Cartilage and Chondrocytes

Xinfang Li, Hassan Afif, Saranette Cheng, Johanne Martel-Pelletier, Jean-Pierre Pelletier, Pierre Ranger, and Hassan Fahmi.

ABSTRACT.

Objective. Elevated production of prostaglandin E_2 (PGE_2) plays an important role in the pathogenesis of arthritis. Recently, an inducible microsomal prostaglandin E synthase-1 (mPGES-1) was identified. This enzyme is functionally coupled with cyclooxygenase-2 (COX-2) and converts the COX product PGH_2 to PGE_2. In the present study, we analyzed the expression of mPGES-1 in human normal and osteoarthritic (OA) cartilage and determined the effect of different inflammatory agonists on the expression of mPGES-1 in OA chondrocytes.

Methods. The expression of mPGES-1 mRNA and protein in cartilage was determined by quantitative real-time reverse transcriptase-polymerase chain reaction and immunohistochemistry, respectively. OA chondrocytes were treated with different inflammatory agents and mPGES-1 protein expression was evaluated by Western blot. Activation of the mPGES-1 promoter was assessed in transient transfection experiments.

Results. Levels of mPGES-1 mRNA and protein were markedly elevated in OA versus normal cartilage. Treatment of chondrocytes with interleukin-1β (IL-1β) induced the expression of mPGES-1 protein in a dose- and time-dependent manner. This appears to occur at the transcriptional level, as IL-1β induced the expression of mPGES-1 mRNA and the activity of this gene promoter. Tumor necrosis factor-α (TNF-α) and IL-17 also up-regulated the expression of mPGES-1 protein and displayed a synergistic effect with IL-1β. Peroxisome proliferator-activated receptor γ ligands, 15-deoxy-$\Delta^{12,14}$-

prostaglandin J_2 and troglitazone, inhibited IL-1β-induced mPGES-1 protein expression, an effect that was reversed by exogenous PGE_2.

Conclusion. This study shows that mPGES-1 expression is up-regulated in OA versus normal cartilage and that pro-inflammatory cytokines increased mPGES-1 expression in chondrocytes. These data suggest that mPGES-1 may prove to be an interesting therapeutic target for controlling PGE_2 synthesis. (J Rheumatol 2005;32:887-95)

Key Indexing Terms:
Microsomal prostaglandin E synthase-1, cartilage, chondrocytes, osteoarthritis.

From the Osteoarthritis Research Unit, Centre hospitalier de l'Université de Montréal, Hôpital Notre-Dame, Montréal, Québec, Canada

Sources of support: This work was supported by the Canadian Institutes of Health Research (CIHR) Grant IMH-63168 and the Fonds de Recherche en santé du Québec (FRSQ) Subvention d'Établissement de Jeune Chercheur #JC2836. S.C is supported by a fellowship from the CIHR Training on Mobility and Posture Deficiencies (MENTOR). H. F. is a research Scholar of FRSQ.

X. Li, MSc, H. Afif, PhD, S. Cheng, MSc, J. Martel-Pelletier, PhD, J-P Pelletier, MD, H. Fahmi, PhD. Osteoarthritis Research Unit, Centre hospitalier de l'Université de Montréal. P. Ranger, MD, Hopital Sacré-Coeur.

Address correspondence and reprint requests to: Hassan Fahmi, PhD, Osteoarthritis Research Unit, Centre hospitalier de l'Université de Montréal, Hôpital Notre-Dame, 1560 rue Sherbrooke est, Montréal, Québec, Canada H2L 4M1. Tel. 514-890-8000, ext. 28910, Fax 514-412-7583, E-mail: [redacted]

INTRODUCTION

Biochemical, genetic, and clinical evidence indicate that prostaglandin (PG) E_2 plays a critical role in inflammation and in the pathophysiology of articular joint diseases, such as rheumatoid arthritis (RA) and osteoarthritis (OA). For example, arthritic joint tissues produce large quantities of PGE_2 (1). Treatment with neutralizing anti-PGE_2 antibodies prevents acute and chronic inflammation in a rat adjuvant arthritis model (2). More direct evidence for the role of PGE_2 in arthritis have been provided by gene targeting studies. Genetic disruption of either the PGE_2 receptor EP4 (3), or cyclooxygenase-2 (4), one of the key enzymes in PGE_2 biosynthesis, reduced the incidence and severity of collagen-induced arthritis in mice. These animals showed reduced inflammation and less cartilage and bone destruction. The role of PGE_2 in arthritis is also supported by effective suppression of pain and inflammatory responses in arthritis by nonsteroidal antiinflammatory drugs (NSAIDs) that reduce PGE_2 biosynthesis (5, 6).

Chondrocytes are a major source of PGE_2 in the joint; the production of this prostanoid can be induced by proinflammatory cytokines, mitogens, mechanical stress, and trauma (5, 7, 8). The synthesis of PGE_2 from arachidonic acid (AA) requires two enzymes acting sequentially. Cyclooxygenases (COXs) catalyze the conversion of AA to the intermediate prostanoid PGH_2. Two isoforms of the COX enzyme have been identified: COX-1 is constitutively expressed in most tissues; whereas COX-2, is induced by various stimuli, including lipopolysaccharide (LPS), growth factors, and proinflammatory cytokines (reviewed in refs. (5, 9)). Subsequently, PGE synthase (PGES) convert COX-derived PGH_2 into PGE_2. At least three distinct PGES isoforms have been identified (10). Cytosolic PGES (cPGES), which is identical to the heat shock protein 90-associated protein p23, is ubiquitously and constitutively expressed and displays functional coupling with COX-1. In contrast, microsomal PGES-1 (mPGES-1), originally designated microsomal glutathione S-transferase 1-like 1 (MGST1-L1), is an inducible enzyme that exhibits preferential functional coupling with COX-2. The most recently identified isoform, mPGES-2, is ubiquitously expressed in diverse tissues, but its function and regulation remain obscure (10).

The up-regulation of mPGES-1 expression has been reported in conditions in which PGE_2 has been implicated, such as arthritis (11) and studies with mPGES-1-deficient mice have shown that induced PGE_2 synthesis is largely dependent on this enzyme (12, 13). Pro-inflammatory cytokines IL-1β and TNF-α have been shown to induce mPGES-1 expression in several tissues and cell types, including synovial fibroblasts and osteoblasts (14, 15). However, little is known about the expression and regulation of mPGES-1 in cartilage.

In order to better understand the regulation of PGE_2 production in joint tissues, we analyzed mPGES-1 expression in normal and OA cartilage. Furthermore, we explored the effect of different inflammatory agonists on the expression of mPGES-1 in OA chondrocytes.

MATERIALS AND METHODS

Reagents. Recombinant human (rh) IL-1β was obtained from Genzyme (Cambridge, MA), rhTNF-α and rhIL-17 were from R&D Systems (Minneapolis, MN). 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), troglitazone, Wy14643, and PGE_2 were from Cayman Chemical Co (Ann Arbor, MI). BRL 49653 was from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM), penicillin and streptomycin, fetal calf serum (FCS), and TRIzol® reagent were from Invitrogen (Burlington, ON, Canada). All other chemicals were purchased from either Bio-Rad (Mississauga, ON, Canada) or Sigma-Aldrich Canada (Oakville, ON, Canada).

Specimen selection and chondrocyte culture. Human normal cartilage (femoral condyles) was obtained at necropsy, within 12 hours of death, from donors with no history of arthritic diseases (n=7, mean ± SEM age: 61 ± 15 years). To ensure that only normal tissue was used, cartilage specimens were thoroughly examined both macroscopically and microscopically. Only those with neither alterations were further processed. Human OA cartilage was obtained from patients undergoing total knee replacement (n=25, mean ± SEM age: 64 ± 14 years). All OA patients were diagnosed on criteria developed by the American College of Rheumatology Diagnostic

Subcommittee for OA. At the time of surgery, the patients had symptomatic disease requiring medical treatment in the form of NSAIDs or selective COX-2 inhibitors. Patients who had received intraarticular injections of steroids were excluded. The Clinical Research Ethics Committee of Notre-Dame Hospital approved the study protocol and the use of human tissues.

Chondrocytes were released from cartilage by sequential enzymatic digestion as previously described (16). Briefly, this consisted of 2 mg/ml pronase for 1 hour followed by 1 mg/ml collagenase for 6 hours (type IV; Sigma-Aldrich) at 37°C in DMEM and antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin). The digested tissue was briefly centrifuged and the pellet was washed. The isolated chondrocytes were seeded at high density in tissue culture flasks and cultured in DMEM supplemented with 10% heat-inactivated FCS. At confluence, the chondrocytes were detached, seeded at high density, and allowed to grow in DMEM, supplemented as above. The culture medium was changed every second day, and 24 hours before the experiment the cells were incubated in fresh medium containing 0.5% FCS. Only first passaged chondrocytes were used.

Immunohistochemistry. Cartilage specimens were processed for immunohistochemistry as previously described (16). The specimens were fixed in 4% paraformaldehyde and embedded in paraffin. Sections (5 µm) of paraffin-embedded specimens were deparaffinized in toluene, and dehydrated in a graded series of ethanol. The specimens were then preincubated with chondroitinase ABC (0.25 U/ml in PBS pH 8.0) for 60 minutes at 37°C, followed by a 30 min incubation with Triton X-100 (0.3%) at room temperature. Slides were then washed in PBS followed by 2% hydrogen peroxyde/methanol for 15 min. They were further incubated for 60 min with 2% normal serum (Vector Laboratories, Burlingame, CA) and overlaid with primary antibody for 18 h at 4 °C in a humidified chamber. The antibody was a rabbit polyclonal anti-human mPGES-1 (Cayman), used at 10 µg/ml. Each slide was washed 3 times in PBS pH 7.4 and stained using the avidin-biotin complex method (Vectastain ABC kit; Vector Laboratories). The color was developed with 3,3'-diaminobenzidine (DAB) (Vector Laboratories) containing hydrogen peroxyde. The slides were counterstained with

eosin. The specificity of staining was evaluated by using antibody that had been preadsorbed (1 hour, 37°C) with a 20-fold molar excess of the specific corresponding peptide, and by substituting the primary antibody with non-immune rabbit IgG (Chemicon, Temecula, CA, used at the same concentration as the primary antibodies). The evaluation of positive-staining chondrocytes was performed using our previously published method (16). For each specimen, 6 microscopic fields were examined under 40X magnification. The total number of chondrocytes and the number of chondrocytes staining positive were evaluated and results were expressed as the percentage of chondrocytes staining positive (cell score).

RNA extraction and reverse transcriptase-polymerase chain reaction. Total RNA from homogenised cartilage or stimulated chondrocytes was isolated using the TRIzol® reagent (Invitrogen) according to the manufacturer's instructions. To remove contaminating DNA, isolated RNA was treated with RNase-free DNase I (Ambion, Austin, TX). The RNA was quantitated using the RiboGreen RNA quantitation kit (Molecular Probes, Eugene, OR), dissolved in diethylpyrocarbonate (DEPC)-treated-H_2O and stored at -80°C until use. One microgram of total RNA was reverse-transcribed using Moloney Murine Leukemia Virus reverse transcriptase (Fermentas, Burlington, ON, Canada) as detailed in the manufacturer's guidelines. One fiftieth of the reverse transcriptase reaction was analyzed by real-time quantitative PCR as described below. The following primers were used: mPGES-1, sense 5'-GAAGAAGGCCTTTGCCAAC-3' and antisense 5'-GGAAGACCAGGAAGTGCATC-3'; cPGES, sense 5'-GCAAAGTGGTACGATCGAAGG-3' and antisense 5'-TGTCCGTTCTTTTATGCTTGG-3'; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), sense 5'-CAGAACATCATCCCTGCCTCT-3' and antisense 5'-GCTTGACAAAGTGGTCGTTGAG -3'.

Real-time Quantitative PCR. Quantitative PCR analysis was performed in a total volume of 50 µl containing template DNA, 200 nM of sense and antisense primers, 25 µl of SYBR® Green master mix (Qiagen, Mississauga, ON, Canada) and uracil-N-

glycosylase (UNG, 0.5 Unit, Epicentre Technologies, Madison, WI). After incubation at 50°C for 2 minutes (UNG reaction), and at 95°C for 10 min (UNG inactivation and activation of the AmpliTaq Gold enzyme), the mixtures were subjected to 40 amplification cycles (15 s at 95° C for denaturation and 1 min for annealing and extension at 60° C). Incorporation of SYBR® Green dye into PCR products was monitored in real time using a GeneAmp 5700 Sequence detection system (Applied Biosystems, Foster City, CA) allowing determination of the threshold cycle (C_T) at which exponential amplification of PCR products begins. After PCR, dissociation curves were generated with one peak, indicating the specificity of the amplification. A threshold cycle (C_T value) was obtained from each amplification curve using the software provided by the manufacturer (Applied Biosystems). Preliminary experiments showed that the amplification efficiency of cPGES, mPGES-1, and GAPDH were similar.

Relative amounts of mRNA in normal and OA cartilage were determined using the standard curve method. Serial dilutions of internal standards (plasmids containing cDNA of target genes) were included in each PCR run, and standard curves for the target gene and for GAPDH were generated by linear regression using log (C_T) versus log (cDNA relative dilution). The C_T were then converted to number of molecules. Relative mRNA expression in cultured chondrocytes was determined using the $\Delta\Delta C_T$ method, as detailed in the manufacturer's guidelines (Applied Biosystems). A ΔC_T value was first calculated by subtracting the C_T value for the housekeeping gene GAPDH from the C_T value for each sample. A $\Delta\Delta C_T$ value was then calculated by subtracting the ΔC_T value of the control (unstimulated cells) from the ΔC_T value of each treatment. Fold changes compared with the control were then determined by raising 2 to the $\Delta\Delta C_T$ power. Each PCR reaction generated only the expected specific amplicon as shown by the melting-temperature profiles of the final product and by gel electrophoresis of test PCR reactions. Each PCR was performed in triplicate on two separate occasions for each independent experiment.

PGE_2 assay. At the end of the incubation period, the culture medium was collected and stored at - 80°C. Levels of PGE_2 were determined using a PGE_2 enzyme immunoassay

kit from Cayman Chemical. The detection limit and sensitivity was 9 pg/ml. All assays were performed in duplicate.

Plasmids and transient transfection. The luciferase reporter constructs pmPGES-1-Luc and pcPGES-Luc were kindly provided by Dr. Terry J. Smith (University of California, Los Angeles) (17). pmPGES-1-Luc contains a 510-bp fragment of the human mPGES-1 promoter spanning -538 to -28. pcPGES-Luc contains a 1824-bp fragment of the human cPGES promoter spanning -1893 to -69. β-galactosidase reporter vector under the control of SV40 promoter (pSV40-β-galactosidase) was from Promega (Madison, WI). Transient transfection experiments were performed using FuGene-6 (1 μg DNA :4 μl FuGene 6) (Roche Applied Science, Laval, Quebec, Canada) according to the manufacturer's recommended protocol. Briefly, chondrocytes were seeded and grown to 50-60% confluence. The cells were transfected with 1 μg of the reporter construct and 0.5 μg of the internal control pSV40-β-galactosidase (Promega). Six hours later, the medium was replaced with DMEM containing 1% FCS. The next day, the cells were treated for 18 h with or without IL-1β. After harvesting, luciferase activity was determined and normalized to β-galactosidase activity (16).

Western blot analysis. Chondrocytes were lysed in ice-cold lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM EDTA, 1 mM PMSF, 10 μg/ml each of aprotinin, leupeptin, and pepstatin, 1 % NP-40, 1 mM Na_3VO_4, and 1 mM NaF). Lysates were sonicated on ice and centrifuged at 12000 rpm for 15 min. The protein concentration of the supernatant was determined using the bicinchoninic acid method (Pierce, Rockford, IL). Ten μg of total cell lysate was subjected to SDS-polyacrylamide gel electrophoresis and electrotransferred to a nitrocellulose membrane (Bio-Rad). After blocking in 20 mM Tris-HCl pH 7.5 containing 150 mM NaCl, 0.1 % Tween 20, and 5 % (w/v) non-fat dry milk, blots were incubated overnight at 4°C with primary antibodies and washed with a tris buffer (Tris-buffered saline (TBS) pH 7.5, with 0.1 % Tween 20). The blots were then incubated with horseradish peroxidase-conjugated secondary antibody (Pierce), washed again, incubated with SuperSignal Ultra

Chemiluminescent reagent (Pierce), and, finally, exposed to Kodak X-Omat film (Eastman Kodak Ltd, Rochester, NY).

Statistical analysis. Data are expressed as the mean ± SEM. Statistical significance was assessed by the 2-tailed Student t-test. p values less than 0.05 were considered significant.

RESULTS

Increased expression of mPGES-1 in OA cartilage. We first analyzed the levels of mPGES-1 mRNA in normal (n=7) and OA (n=8) cartilage using real-time quantitative RT-PCR. As shown in Figure 1A, levels of mPGES-1 expression in cartilage from OA patients were 2.8-fold higher compared with those from normal cartilage ($p < 0.05$). In contrast to mPGES-1, there was no statistically significant difference in the level of cPGES expression between OA and normal cartilage (Fig. 1B).

To examine whether mPGES-1 protein is also expressed in cartilage, normal (n = 5) and OA (n = 5) cartilage were processed for immunohistochemical analysis. In normal cartilage, the positive immunostaining for mPGES-1 was located only in a few chondrocytes in the superficial layer (mean ± SEM 9.1 ± 0.6%) (Fig. 2A). In contrast, the cell score was higher in OA cartilage (mean ± SEM 24.4 ± 1.8%) than it was in normal cartilage (Fig. 2B). Statistical evaluation of the cell score for mPGES-1 indicated significant differences between normal and OA cartilage ($p<0.001$). The specificity of staining was confirmed by immunohistochemical staining using an anti-mPGES-1 antibody that had been preadsorbed with the peptide antigen (Fig. 2C) or non-immune rabbit IgG at the same concentration (data not shown). These observations demonstrate an up-regulation of mPGES-1 expression in OA cartilage.

Induction of mPGES-1 expression by IL-1β in chondrocytes. To explore the mechanisms by which mPGES-1 is regulated in cartilage, we examined the effect of IL-1β, a key mediator in the pathogenesis of arthritis, on both the expression of mPGES-1 and the production of PGE_2 by OA chondrocytes. Under basal culture conditions, OA chondrocytes express low levels of mPGES-1 protein. Treatment with IL-1β (100

pg/ml) enhanced the expression of mPGES-1 protein in a time-dependent manner (Fig. 3A). The level of mPGES-1 expression started to increase 6 h post-stimulation with IL-1β and reached the maximum at 24 h. The increased expression of mPGES was sustained for at least 48 h. The induction of mPGES-1 protein expression was also dose-dependent (Fig. 3B). The level of mPGES-1 was increased at IL-1β concentrations as low as 1 pg/ml and reached a maximum at 100 pg/ml. In concert with the effects on mPGES-1 expression, IL-1β stimulated PGE_2 production in a time- and dose-dependent manner (Fig. 3, lower panels). In contrast, the level of cPGES was not altered as a consequence of IL-1β treatment.

IL-1β induces mPGES-1 expression at the transcriptional level. To further elucidate the mechanism responsible for the up-regulation of mPGES-1 protein, we analyzed the effect of IL-1β on the expression of mPGES-1 mRNA. Chondrocytes were treated with increasing concentrations of IL-1β for 12 h, and specific mRNA for mPGES-1 and cPGES were quantified by real-time RT-PCR. IL-1β-induced changes in gene expression were expressed as -fold over control (untreated cells) after normalizing to the internal control GAPDH. Results showed that IL-1β induced a dose-dependent increase in mPGES-1 mRNA expression, but had no effect on the levels of cPGES mRNA (Fig. 4A).

To determine whether changes in mRNA levels can be ascribed to changes in promoter activity, chondrocytes were transiently transfected with the human mPGES-1 or cPGES promoter-luciferase reporter genes. Treatment of transfected cells with increasing concentrations of IL-1β led to a dose-dependent increase of the mPGES-1 promoter activity (Fig. 4B). In contrast, IL-1β had no significant effect on the cPGES promoter activity. These data are consistent with the regulation of mPGES-1 expression by IL-1β being at the level of transcription.

Effect of the combination of IL-1β, TNF-α and IL-17 on mPGES-1 expression. The pro-inflammatory cytokines TNF-α and IL-17 are also implicated in the pathogenesis of arthritis, and are potent inducers of COX-2 expression and PGE_2 production in articular

chondrocytes (18, 19). Therefore, we examined the effect of both cytokines on mPGES-1 expression. Chondrocytes were stimulated with increasing concentrations of IL-17 (0.5-1000 ng/ml) or TNF-α (0.5-10000 pg/ml) for 24 h and mPGES-1 protein expression was evaluated by Western blot analysis. As shown in Figure 5A and 5B, treatment with TNF-α or IL-17 induced mPGES-1 expression and PGE_2 production in a dose-dependent manner. At optimal concentrations the effect of IL-1β (100 pg/ml) was more potent than that of IL-17 (1000 ng/ml) or of TNF-α (10 ng/ml).

Previous studies have demonstrated that low concentrations of IL-1β, TNF-α and IL-17 were synergistic in a number of systems (20-24). Therefore, we examined the effect of different combinations of these cytokines on mPGES-1 expression and PGE_2 production. At a lower concentration, IL-1β (0.1 pg/ml), IL-17 (0.5 ng/ml) or TNF-α (0.5 pg/ml) alone had little or no effect on mPGES-1 expression and PGE_2 production. Each combination of two cytokines resulted in a marked increase of mPGES-1 expression versus either cytokine alone, indicating a synergistic effect (Fig. 5C). The combination of either IL-1β and TNF-α or IL-1β and IL-17 resulted in a greater effect than IL-17 and TNF-α. In addition, the combination of three cytokines led to a more potent effect versus the combination of any 2 of the cytokines (Fig. 5C). These findings indicate that low levels of cytokines can act in combination to up-regulate the expression of mPGES-1.

Peroxisome proliferator-activated receptorγ (PPARγ) ligands inhibited IL-1β-induced mPGES-1 expression. PPARγ ligands have been shown to inhibit the expression of a number of genes involved in the pathogenesis of arthritis (25). To assess the effect of these molecules on mPGES-1 expression in chondrocytes, we first examined the natural PPARγ ligand, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2). Chondrocytes were stimulated with IL-1β (100 pg/ml) in the absence or presence of increasing concentrations of 15d-PGJ_2, and the expression of mPGES-1 was evaluated by Western blotting. As shown in Figure 6A, 15d-PGJ_2 dose-dependently prevented IL-1β-induced mPGES-1 expression. Troglitazone, a synthetic and selective PPARγ ligand, also inhibited IL-1β-induced mPGES-1 expression (Fig. 6B). In contrast, the

PPARα ligand, Wy14643, did not affect IL-1β-induced mPGES expression (Fig. 6C). cPGES expression was not affected by these treatments (Fig. 6, lower panels). Taken together, these data suggest that 15d-PGJ2, prevented IL-1β-induced mPGES-1 expression, at least in part, through a PPARγ-dependent mechanism.

PPARγ ligands inhibited IL-1β-induced mPGES-1 expression is alleviated by PGE_2. Next, we evaluated the role of PGE_2, the end product of mPGES-1, in the repressing effect of PPARγ ligands. Chondrocytes were preincubated with increasing concentrations of PGE_2 (0.01-1 μM) for 30 min, prior to the addition of 15d-PGJ_2 (20 μM) or troglitazone (50 μM), and were subsequently stimulated with IL-1β (100 pg/ml) for 24 h. Western blot analysis revealed that PGE_2 dose-dependently alleviated the suppressive effect of 15d-PGJ_2 (Fig. 7A) or troglitazone (Fig. 7B) on IL-1β-induced mPGES-1 expression. Of note, PGE_2 alone had no significant effect on mPGES-1 expression (Fig. 7A and B, last three lanes). As expected, the level of cPGES expression was not affected by these treatments.

DISCUSSION

It is well established that increased production of PGE_2 plays a central role in the pathogenesis of arthritis, and inhibitors of PGE_2 synthesis are widely used in the treatment of OA and RA (5, 26). PGE_2 biosynthesis from AA is controlled by two rate-limiting enzymatic reactions. The first step is catalyzed by COX, which transforms AA into the unstable metabolite PGH_2. The second step is catalyzed by PGES, which converts PGH_2 into PGE_2. Several PGES were identified, among which mPGES-1 has been shown to be functionally coupled with COX-2 and to be up-regulated by pro-inflammatory stimuli in several cell types and tissues (10). However, little is known about the expression and regulation of mPGES-1 in human cartilage.

In this study, we showed that human cartilage also expresses mPGES-1. Using real time quantitative RT-PCR, we found that mPGES-1 mRNA expression was elevated in OA cartilage when compared with normal cartilage. Immunohistochemical analysis corroborates these findings, showing higher mPGES-1-positive cells in OA versus normal cartilage. This is similar to the results of two recent studies showing that

mPGES-1 is overexpressed in OA cartilage (27, 28). In our studies, the positive immunoreactive staining for mPGES-1 was located mainly in chondrocytes in the superficial layers. Interestingly, IL-1β, one of the most important mediators involved in articular inflammation and degradation processes, has been shown to accumulate in these zones (29, 30). This suggests that IL-1β could be a key mediator of mPGES-1 expression in chondrocytes. Indeed, cell culture experiments demonstrated that IL-1β induced mPGES-1 protein expression in a dose-dependent manner. Time course analysis showed that mPGES-1 protein started to increase 6 h post-stimulation with IL-1β and remained elevated even 48 h after IL-1β stimulation. This is in contrast to other IL-1β-induced genes in chondrocytes, the expression of which is rapidly induced (2-3 h), reaching a maximum at 8 h, and then gradually decreased to reach basal level at 24-36 h. These differences in the kinetics of induction suggest that the mechanisms controlling the expression of these genes are not identical.

The up-regulation of mPGES-1 expression by IL-1β occurred, at least in part, at the transcriptional level, as detemined by real-time quantitative RT-PCR and transient transfection experiments. With regard to the mechanism by which IL-1β induces mPGES-1 transcription, it is known that the human mPGES-1 promoter contains several potential transcription factor-binding sites, including two GC boxes, two Barbie boxes, and an aryl hydrocarbon response element (31). Naraba et al (32) showed that the binding of Egr-1, an inducible transciption factor, to the proximal GC box plays an essential role in the induction of mPGES-1 in macrophages and osteoblastic cells. This is consistent with other reports that Egr-1 is important for mPGES-1 transcription in human colonocytes (33) and synovial fibroblasts (34). Although we have not investigated Egr-1, it seems likely that this transcription factor may also play an important role in induced mPGES-1 gene expression in chondrocytes.

The pro-inflammatory cytokines TNF-α and IL-17 have also been implicated in the pathogenesis of arthritis. These mediators are present at elevated levels in articular joint tissues and are believed to induce their effect through enhancing the production of a number of inflammatory and catabolic factors (18, 19, 35, 36). Like IL-1β, we found that TNF-α and IL-17 also induced mPGES-1 expression. At optimal concentrations, the effect of IL-17 or TNF-α on mPGES-1 expression and PGE2 production was less

potent than that of IL-1β. Recently, numerous studies have described synergistic or additive effects between IL-1β, TNF-α and IL-17 in many systems. Katz et al (20) demonstrated in human OA synovial fibroblasts that the combination of IL-17 and TNF-α synergistically stimulated the production of IL-1, IL-6, and IL-8. Chabaud et al (21) found that combinations of IL-1β, TNF-α and IL-17 were synergistic on the production of macrophage inflammatory protein-3α by RA synovial fibroblasts. Berenbaum et al (24) showed that IL-1β and TNF-α synergize to induce PGE_2 production and COX-2 expression in rabbit articualr chondrocytes. Synergy between IL-1β, TNF-α and IL-17 on nitric oxide (NO) and PGE_2 production by explants of human OA knee menisci was demonstrated by LeGrand et al (22). Here, we extend these findings by showing that combinations of IL-1, TNF-α and IL-17 were synergistic on the induction of mPGES-1 protein expression. Moreover, the effect of the combination of three cytokines was stronger than that of each of the two cytokines, suggesting that the combination of cytokines may be of importance in the increased expression of mPGES-1. As expected, the level of cPGES expression was not altered by these treatments. mPGES-2 protein was also present in cultured chondrocytes, but its expression did not change with any of the treatments used in our studies (data not shown). This is consistent with other reports showing that the expression of mPGES-2 is not affected by proinflammatory stimuli in several cell types, including chondrocytes (27, 37).

Recently, numerous studies have shown that PPARγ ligands inhibit the expression of several genes involved in the pathogenesis of arthritis. For example, PPARγ ligands prevent the expression of IL-1, IL-6, and TNF-α in activated monocytes/macrophages, as well as that of collagenase-1 in synovial fibroblasts, and collagenase-3 and the inducible NO synthase (iNOS) in chondrocytes (25, 38). PPARγ ligands were also shown to inhibit the induction of PGE_2 production in a number of experimental systems (34, 39, 40). In the present study, we showed that the PPARγ ligands 15d-PGJ_2 and troglitazone, but not the PPARα ligand Wy14643, repressed IL-1β-induced mPGES-1 expression. Interestingly, treatment with PGE_2 restored the expression of mPGES-1. However, PGE_2 had no significant effect on unstimulated

mPGES-1 expression, indicating that additional signals are provided by IL-1β stimulation, that PGE_2 alone cannot provide. These data also suggest that the up-regulation of mPGES-1 is dependent, at least in part, on PGE_2 production. This suggestion is supported by the findings that inhibition of IL-1β-induced mPGES expression by NSAIDs is restored by exogenous AA and PGE_2 (41, 42).

In addition to its pro-inflammatory effects, the elevated biosynthesis of PGE_2 has been associated with the erosion of cartilage and juxta-articular bone. PGE_2 can contribute to joint tissue damage by inhibiting collagen and proteoglycan synthesis, promoting the production of matrix metalloproteases, and suppressing the synthesis of tissue inhibitor of metalloproteases (5, 43). In addition, PGE_2 triggers osteoclastic bone resorption (5). The up-regulation of mPGES-1 in cartilage from OA patients suggests its involvement in local increased PGE_2 production and tissular destruction. This is supported by the findings that the degradation of cartilage and bone were reduced in mPGES-1 deficient mice (13, 44).

In conclusion, the data presented in this report show that the expression of mPGES-1 is up-regulated in OA cartilage. The pro-inflammatory cytokines IL-1, TNF-α, and IL-17 may be responsible for this up-regulation. Combined with results from previous studies showing a critical role of mPGES-1 in the synthesis of PGE_2 and the pathogenesis of arthritis (11-13, 44, 44). these data suggest that mPGES-1 constitutes a novel therapeutic target in the treatment of arthritis and possibly that of other diseases in which increased production of PGE_2 is implicated.

ACKNOWLEDGEMENTS

We thank Dr. T. J. Smith from the University of California for kindly providing us with the human mPGES-1 and cPGES-1 promoters. We are also grateful to C. Boileau, M. Boily, F. Mineau, and G. Tardif for their helpful suggestions.

REFERENCES

1. Amin AR, Attur M, Patel RN, Thakker GD, Marshall PJ, Rediske J, et al: Superinduction of cyclooxygenase-2 activity in human osteoarthritis-affected cartilage. Influence of nitric oxide. J Clin Invest 1997;99:1231-1237.

2. Portanova JP, Zhang Y, Anderson GD, Hauser SD, Masferrer JL, Seibert K, et al: Selective neutralization of prostaglandin E2 blocks inflammation, hyperalgesia, and interleukin 6 production in vivo. J Exp Med 1996;184:883-891.

3. McCoy JM, Wicks JR, Audoly LP: The role of prostaglandin E2 receptors in the pathogenesis of rheumatoid arthritis. J Clin Invest 2002;110:651-658.

4. Myers LK, Kang AH, Postlethwaite AE, Rosloniec EF, Morham SG, Shlopov BV, et al: The genetic ablation of cyclooxygenase 2 prevents the development of autoimmune arthritis. Arthritis Rheum 2000;43:2687-2693.

5. Martel-Pelletier J, Pelletier JP, Fahmi H: Cyclooxygenase-2 and prostaglandins in articular tissues. Semin Arthritis Rheum 2003;33:155-167.

6. Crofford LJ: Specific cyclooxygenase-2 inhibitors: what have we learned since they came into widespread clinical use? Curr Opin Rheumatol 2002;14:225-230.

7. Chrisman OD, Ladenbauer-Bellis IM, Panjabi M, Goeltz S: 1981 Nicolas Andry Award. The relationship of mechanical trauma and the early biochemical reactions of osteoarthritic cartilage. Clin Orthop 1981;275-284.

8. Fermor B, Weinberg JB, Pisetsky DS, Misukonis MA, Fink C, Guilak F: Induction of cyclooxygenase-2 by mechanical stress through a nitric oxide-regulated pathway. Osteoarthritis Cartilage 2002;10:792-798.

9. Smith WL, Langenbach R: Why there are two cyclooxygenase isozymes. J Clin Invest 2001;107:1491-1495.

10. Fahmi H: mPGES-1 as a novel target for arthritis. Curr Opin Rheumatol 2004;16:623-627.

11. Mancini JA, Blood K, Guay J, Gordon R, Claveau D, Chan CC, et al: Cloning, expression, and up-regulation of inducible rat prostaglandin e synthase during

lipopolysaccharide-induced pyresis and adjuvant-induced arthritis. J Biol Chem 2001;276:4469-4475.

12. Uematsu S, Matsumoto M, Takeda K, Akira S: Lipopolysaccharide-dependent prostaglandin E(2) production is regulated by the glutathione-dependent prostaglandin E(2) synthase gene induced by the Toll-like receptor 4/MyD88/NF-IL6 pathway. J Immunol 2002;168:5811-5816.

13. Trebino CE, Stock JL, Gibbons CP, Naiman BM, Wachtmann TS, Umland JP, et al: Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. Proc Natl Acad Sci U S A 2003;100:9044-9049.

14. Stichtenoth DO, Thoren S, Bian H, Peters-Golden M, Jakobsson PJ, Crofford LJ: Microsomal prostaglandin E synthase is regulated by proinflammatory cytokines and glucocorticoids in primary rheumatoid synovial cells. J Immunol 2001;167:469-474.

15. Saegusa M, Murakami M, Nakatani Y, Yamakawa K, Katagiri M, Matsuda K, et al: Contribution of membrane-associated prostaglandin E2 synthase to bone resorption. J Cell Physiol 2003;197:348-356.

16. Fahmi H, Di Battista JA, Pelletier JP, Mineau F, Ranger P, Martel-Pelletier J: Peroxisome proliferator--activated receptor gamma activators inhibit interleukin-1beta-induced nitric oxide and matrix metalloproteinase 13 production in human chondrocytes. Arthritis Rheum 2001;44:595-607.

17. Han R, Smith TJ: Cytoplasmic prostaglandin E2 synthase is dominantly expressed in cultured KAT-50 thyrocytes, cells that express constitutive prostaglandin-endoperoxide H synthase-2. Basis for low prostaglandin E2 production. J Biol Chem 2002;277:36897-36903.

18. Shalom-Barak T, Quach J, Lotz M: Interleukin-17-induced gene expression in articular chondrocytes is associated with activation of mitogen-activated protein kinases and NF-kappaB. J Biol Chem 1998;273:27467-27473.

19. Geng Y, Blanco FJ, Cornelisson M, Lotz M: Regulation of cyclooxygenase-2 expression in normal human articular chondrocytes. J Immunol 1995;155:796-801.

20. Katz Y, Nadiv O, Beer Y: Interleukin-17 enhances tumor necrosis factor alpha-induced synthesis of interleukins 1,6, and 8 in skin and synovial fibroblasts: a possible

role as a "fine-tuning cytokine" in inflammation processes. Arthritis Rheum 2001;44:2176-2184.

21. Chabaud M, Page G, Miossec P: Enhancing effect of IL-1, IL-17, and TNF-alpha on macrophage inflammatory protein-3alpha production in rheumatoid arthritis: regulation by soluble receptors and Th2 cytokines. J Immunol 2001;167:6015-6020.

22. LeGrand A, Fermor B, Fink C, Pisetsky DS, Weinberg JB, Vail TP, et al: Interleukin-1, tumor necrosis factor alpha, and interleukin-17 synergistically up-regulate nitric oxide and prostaglandin E2 production in explants of human osteoarthritic knee menisci. Arthritis Rheum 2001;44:2078-2083.

23. Granet C, Miossec P: Combination of the pro-inflammatory cytokines IL-1, TNF-alpha and IL-17 leads to enhanced expression and additional recruitment of AP-1 family members, Egr-1 and NF-kappaB in osteoblast-like cells. Cytokine 2004;26:169-177.

24. Berenbaum F, Jacques C, Thomas G, Corvol MT, Bereziat G, Masliah J: Synergistic effect of interleukin-1 beta and tumor necrosis factor alpha on PGE2 production by articular chondrocytes does not involve PLA2 stimulation. Exp Cell Res 1996;222:379-384.

25. Fahmi H, Pelletier JP, Martel-Pelletier J: PPARgamma ligands as modulators of inflammatory and catabolic responses on arthritis. An overview. J Rheumatol 2002;29: 3-14.

26. Hochberg MC: COX-2: Where are we in 2003? - Be strong and resolute: continue to use COX-2 selective inhibitors at recommended dosages in appropriate patients. Arthritis Res Ther 2003;5:28-31.

27. Kojima F, Naraba H, Miyamoto S, Beppu M, Aoki H, Kawai S: Membrane-associated prostaglandin E synthase-1 is upregulated by proinflammatory cytokines in chondrocytes from patients with osteoarthritis. Arthritis Res Ther 2004;6:R355-365.

28. Masuko-Hongo K, Berenbaum F, Humbert L, Salvat C, Goldring MB, Thirion S: Up-regulation of microsomal prostaglandin E synthase 1 in osteoarthritic human cartilage: critical roles of the ERK-1/2 and p38 signaling pathways. Arthritis Rheum 2004;50:2829-2838.

29. Pelletier JP, Lascau-Coman V, Jovanovic D, Fernandes JC, Manning P, Connor JR, et al: Selective inhibition of inducible nitric oxide synthase in experimental osteoarthritis is associated with reduction in tissue levels of catabolic factors. J Rheumatol 1999;26:2002-2014.

30. Tetlow LC, Adlam DJ, Woolley DE: Matrix metalloproteinase and proinflammatory cytokine production by chondrocytes of human osteoarthritic cartilage: associations with degenerative changes. Arthritis Rheum 2001;44:585-594.

31. Forsberg L, Leeb L, Thoren S, Morgenstern R, Jakobsson P: Human glutathione dependent prostaglandin E synthase: gene structure and regulation. FEBS Lett 2000;471:78-82.

32. Naraba H, Yokoyama C, Tago N, Murakami M, Kudo I, Fueki M, et al: Transcriptional regulation of the membrane-associated prostaglandin E2 synthase gene. Essential role of the transcription factor Egr-1. J Biol Chem 2002;277:28601-28608.

33. Subbaramaiah K, Yoshimatsu K, Scherl E, Das KM, Glazier KD, Golijanin D, et al: Microsomal prostaglandin E synthase-1 is overexpressed in inflammatory bowel disease. Evidence for involvement of the transcription factor Egr-1 a. J Biol Chem 2004;

34. Cheng S, Afif H, Martel-Pelletier J, Pelletier JP, Li X, Farrajota K, et al: Activation of peroxisome proliferator-activated receptor gamma inhibits interleukin-1beta-induced membrane-associated prostaglandin E2 synthase-1 expression in human synovial fibroblasts by interfering with Egr-1. J Biol Chem 2004;279:22057-22065.

35. Honorati MC, Meliconi R, Pulsatelli L, Cane S, Frizziero L, Facchini A: High in vivo expression of interleukin-17 receptor in synovial endothelial cells and chondrocytes from arthritis patients. Rheumatology (Oxford) 2001;40:522-527.

36. Partsch G, Steiner G, Leeb BF, Dunky A, Broll H, Smolen JS: Highly increased levels of tumor necrosis factor-alpha and other proinflammatory cytokines in psoriatic arthritis synovial fluid. J Rheumatol 1997;24:518-523.

37. Giannico G, Mendez M, LaPointe MC: Regulation of the Membrane-Localized Prostaglandin E Synthases mPGES-1 and mPGES-2 in Cardiac Myocytes and Fibroblasts. Am J Physiol Heart Circ Physiol 2004;

38. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK: The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature 1998;391: 79-82.

39. Subbaramaiah K, Lin DT, Hart JC, Dannenberg AJ: Peroxisome proliferator-activated receptor gamma ligands suppress the transcriptional activation of cyclooxygenase-2. Evidence for involvement of activator protein-1 and CREB-binding protein/p300. J Biol Chem 2001;276: 12440-12448.

40. Fahmi H, Pelletier JP, Mineau F, Martel-Pelletier J: 15d-PGJ(2) is acting as a 'dual agent' on the regulation of COX-2 expression in human osteoarthritic chondrocytes. Osteoarthritis Cartilage 2002;10:845-848.

41. Han R, Tsui S, Smith TJ: Up-regulation of prostaglandin E2 synthesis by interleukin-1beta in human orbital fibroblasts involves coordinate induction of prostaglandin-endoperoxide H synthase-2 and glutathione-dependent prostaglandin E2 synthase expression. J Biol Chem 2002;277:16355-16364.

42. Kojima F, Naraba H, Sasaki Y, Beppu M, Aoki H, Kawai S: Prostaglandin E2 is an enhancer of interleukin-1beta-induced expression of membrane-associated prostaglandin E synthase in rheumatoid synovial fibroblasts. Arthritis Rheum 2003;48:2819-2828.

43. Hardy MM, Seibert K, Manning PT, Currie MG, Woerner BM, Edwards D, et al: Cyclooxygenase 2-dependent prostaglandin E2 modulates cartilage proteoglycan degradation in human osteoarthritis explants. Arthritis Rheum 2002;46:1789-1803.

44. Kamei D, Yamakawa K, Takegoshi Y, Mikami-Nakanishi M, Nakatani Y, Oh-Ishi S, et al: Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin e synthase-1. J Biol Chem 2004;279:33684-33695.

Figure 1, Li *et al.*

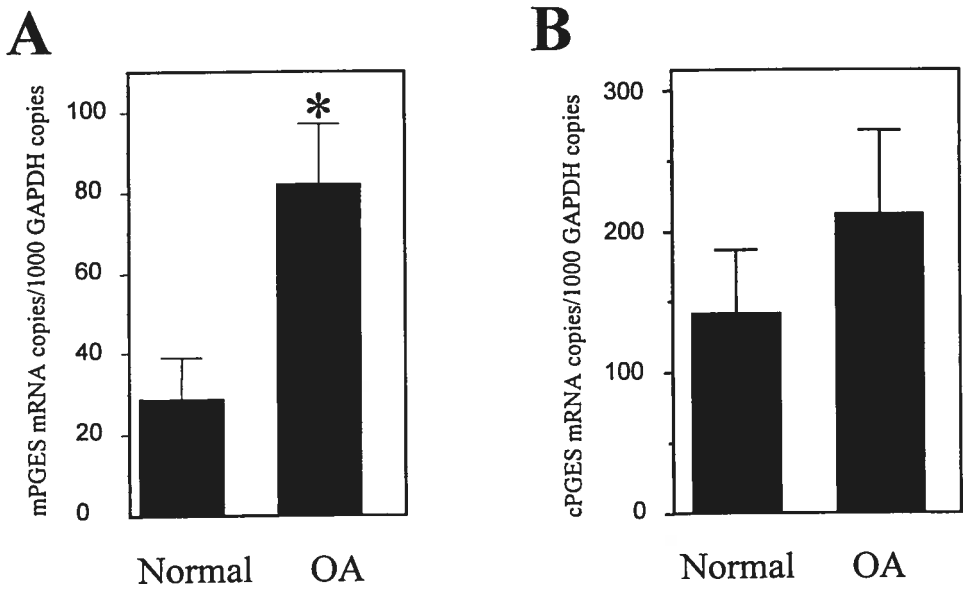


Figure 2, *Li et al.*

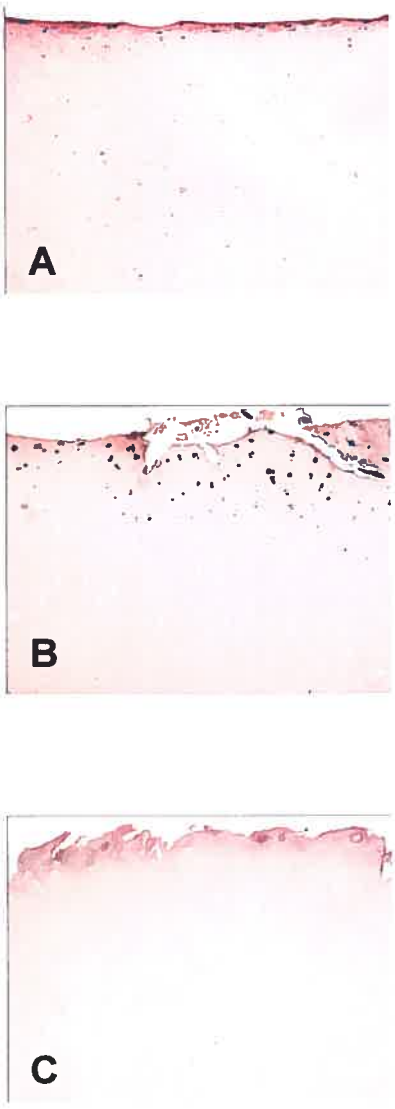


Figure 3, Li *et al.*

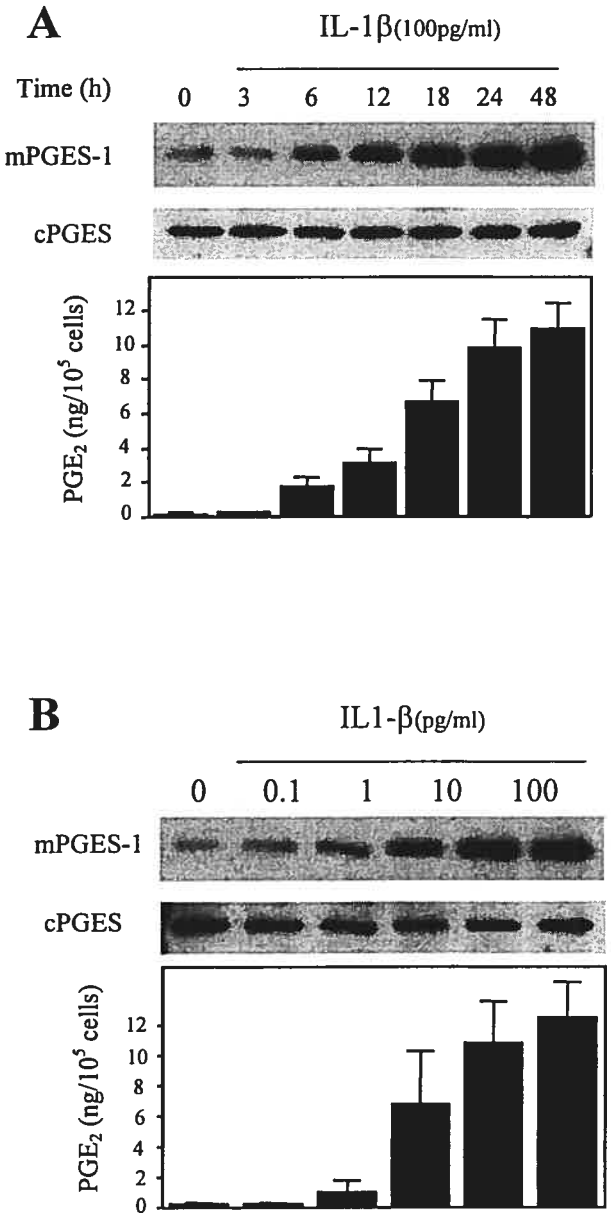


Figure 4, *Li et al.*

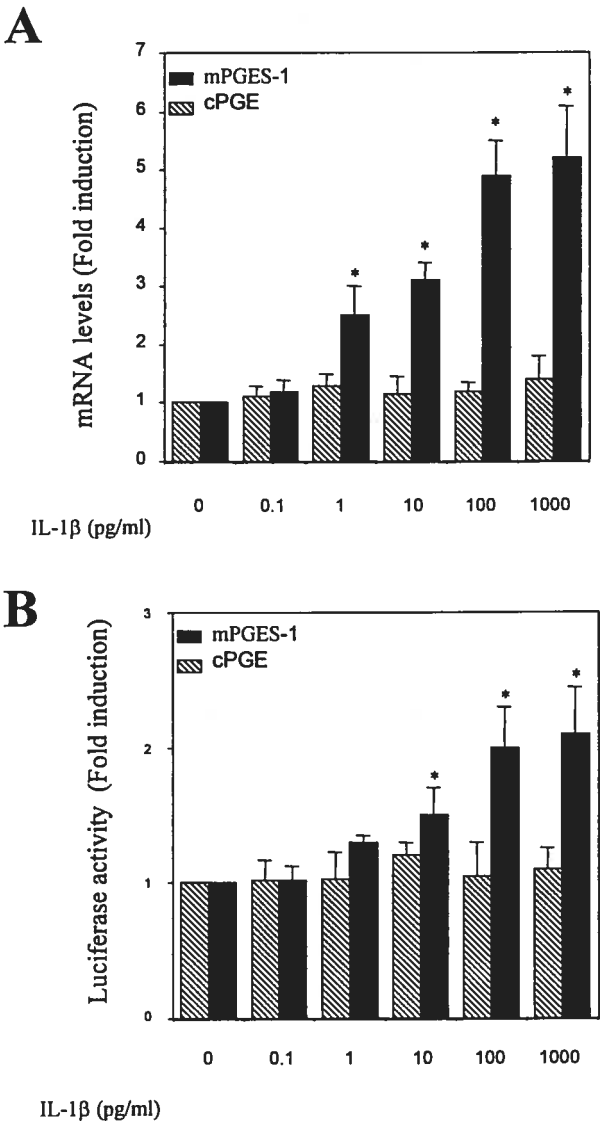


Figure 5, *Li et al.*

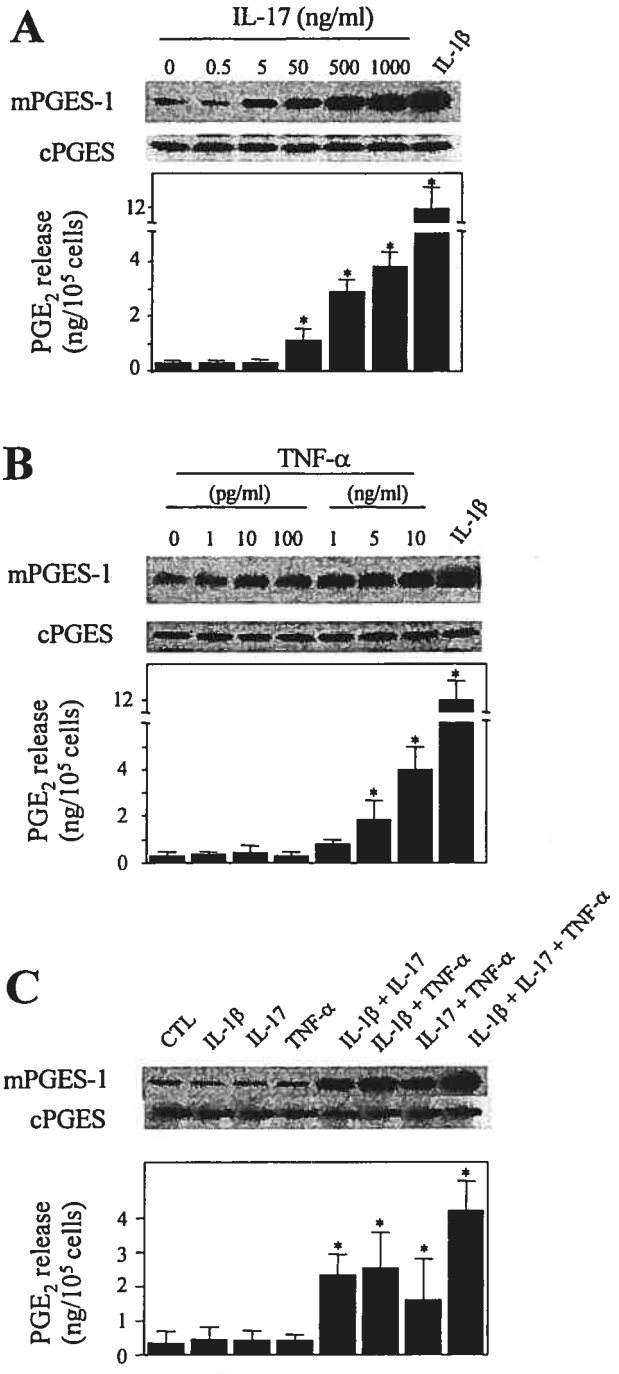


Figure 6, *Li et al.*

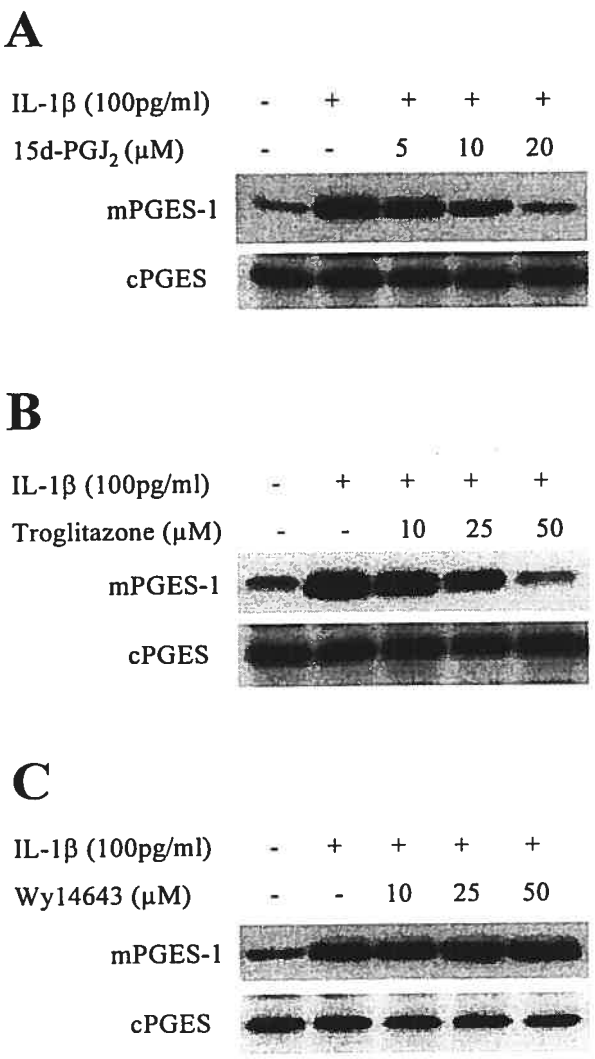
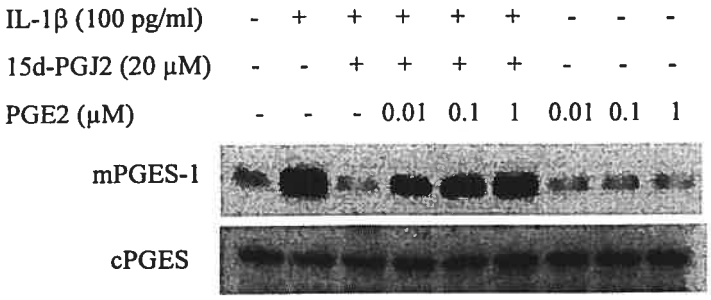


Figure 7, *Li et al.*

A



B

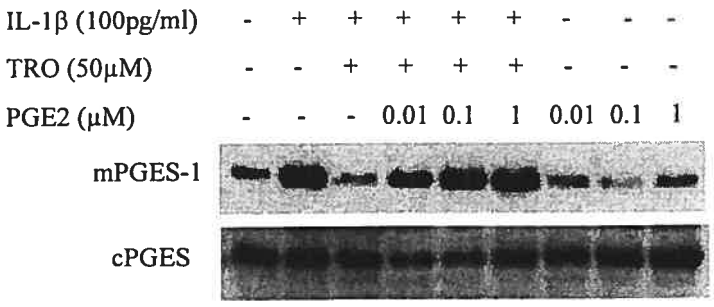


FIGURE LEGENDS

Figure 1. Relative expression of mPGES-1 (A) and cPGES (B) in normal and OA human cartilage. RNA was extracted from normal (n=7) and OA (n=8) cartilage, reverse transcribed into cDNA, and processed for real-time PCR. The threshold cycle values were converted to the number of molecules, as described under Materials and Methods. Data were expressed as copies of gene's mRNA detected per 1000 GAPDH copies. *$p<0.05$ versus normal samples.

Figure 2. Representative immunostaining of human normal (n=5) (A) and OA cartilage (n=5) (B) for mPGES-1. C, OA specimens treated with anti-mPGES-1 antibody that was preadsorbed with a 20-fold molar excess of the blocking mPGES-1 peptide (control for staining specificity). Results are representative of three separate experiments.

Figure 3. Effect of IL-1β on mPGES-1 protein expression in OA chondrocytes. A, cells were treated with 100 pg/ml IL-1β for the indicated time periods. B, Chondrocytes were treated with increasing concentrations of IL-1β for 24 h. Cell lysates were prepared and analyzed for mPGES-1 protein by Western blotting (*upper panels*). The blots were stripped and reprobed with a specific anti-cPGES antibody (*middle panels).* The blots are representative of similar results obtained from 4 independent experiments. In the *lower panels*, conditioned media was collected and analyzed for PGE_2. Results are expressed as the mean ± SEM of 3 independent experiments. *$p<0.05$ compared with unstimulated cells.

Figure 4. IL-1β induced mPGES-1 expression at the transcriptional level. A, Chondrocytes were treated with increasing concentrations of IL-1β for 12 h. Total RNA was isolated; reverse transcribed into cDNA; and mPGES-1, cPGES, and GAPDH mRNAs were quantified using real-time PCR. All experiments were performed in triplicate, and negative controls without template RNA were included in each experiment. B, chondrocytes were co-transfected with 1 μg/well of either the mPGES-1

promoter or the cPGES promoter and 0.5 μg/well of the internal control pSV40-β-galactosidase, using FuGene 6 transfection reagent. The next day, transfected cells were treated with increasing concentrations of IL-1β for 18 h. Luciferase activity values were determined and normalized to β-galactosidase activity. Results are expressed as –fold changes, considering 1 as the value of untreated cells and represent the mean ± SEM of 4 independent experiments. *$p<0.05$ compared with unstimulated cells.

Figure 5. Effect of TNF-α and IL-17 on mPGES-1 protein expression in OA chondrocytes. Cells were treated with increasing concentrations of IL-17 (A), TNF-α(B) or IL-1β (100 pg/ml). C, cells were treated with IL-1β (0.1 pg/ml), IL-17 (0.5 ng/ml), TNF-α (0.5 pg/ml), alone or in combination. After 24 h, cell lysates were prepared and analyzed for mPGES-1 and cPGES proteins by Western blotting. The blots are representative of similar results obtained from four independent experiments. In the *lower panels*, conditioned media was collected and analyzed for PGE_2. Results are expressed as the mean ± SEM of 3 independent experiments. *$p<0.05$ compared with unstimulated cells.

Figure 6. PPARγ ligands inhibited IL-1β-induced mPGES-1 protein expression. Chondrocytes were pretreated with increasing concentrations of 15d-PGJ_2 (A), troglitazone (B) or Wy14643 (C) for 30 min before incubation in the presence of 100 pg/ml IL-1β for 24 h. Cell lysates were prepared and analyzed for mPGES-1 protein by Western blotting. In the *lower panels*, the blots were stripped and reprobed with a specific anti-cPGES antibody. The blots are representative of similar results obtained from four independent experiments.

Figure 7. PPARγ ligands inhibited IL-1β-induced mPGES-1 expression is alleviated by PGE_2. Chondrocytes were pretreated with increasing concentrations of PGE_2 for 30 min. The cells were then treated with or without IL-1β (100 pg/ml) for 24 h in the absence or presence 20 μM 15d-PGJ_2 (A) or 50 μM troglitazone (B). Cell lysates were prepared and analyzed for mPGES-1 protein by Western blotting. In the *lower panels*,

the blots were stripped and reprobed with a specific anti-cPGES antibody. The blots are representative of similar results obtained from four independent experiments.

C. DISCUSSION

Up-regulation of mPGES-1 expression in OA cartilage

Prostaglandins are critical modulators of numerous physiological and pathophysiological conditions including inflammation, immune regulation, cancer, and arthritis. PGE_2 is by far the major prostanoid synthesized in the joint and plays an important role in inflammation and pathogenesis of arthritis and inhibitors of PGE_2 synthesis are widely used in the treatment of arthriris. High levels of PGE_2 are found in serum and synovial fluids from arthritic patients. In addition to its proinflammatory actions, PGE_2 contributes to joint damage by promoting MMP-13 production, osteoclastic bone resorption, and angiogenesis. The critical roles of PGE_2 in the pathology of arthritis were also demonstrated by pharmacological inhibition and gene targeting of COX-2 and the four PGE receptors EP_1-EP_4. With increased understanding of the biosynthesis of PGs, it is generally considered that COX activity is the key step in PG synthesis. However, metabolism of AA by COXs (COX-1 or COX-2) yields only the unstable intermediary PGH_2, which then can be further metabolized into various prostanoids by specific terminal PG synthases, of which PGES enzymes convert PGH_2 to PGE_2 specifically. Among the PGESs, mPGES-1 has been shown to be functionally coupled with COX-2 and to be upregulated by proinflammatory stimuli in several cellular types and tissues including synovial fibroblasts and osteoblasts. Chondrocytes are responsible for maintaing the structural and functional properties of the ECM components of adult articular cartilage. A large number of studies conducted in vitro and in vivo have suggested that cytokines can contribute to the regulation of chondrocytes activity under physiologic and pathologic conditions. Although investigators have used cartilage and isolated chondrocytes from different sources, the results are markably consistent, suggesting that proinflammatory cytokines such as IL-1β and TNF-α contribute to the dysregulation of chondrocyte function that leads to the progressive degradation of the cartilage matrix and loss of joint function. Previous studies have demonstrated that IL-1β stimulates the expression of secreted PLA_2 and COX-2 genes in articular chondrocytes, resulting in increased PGE_2 production (Thomas et al, 2000). In this study, we focused on PGES, the last enzyme in the PGE_2 biosynthesis pathway, and our results showed that mPGES-1, but not cPGES was

overexpressed in OA cartilage and in human articular chondrocytes stimulated with IL-1β. Both mPGES-1, but not cPGES mRNA expression and protein expression were elevated in OA cartilage when compared with normal cartilage. The positive immunoreactive staining for mPGES-1 was located mainly in chondrocytes in the superficial layers. Proinflammatory cytokine IL-1β also has been shown to accumulate in these zones. Previous studies have shown that IL-1β expression is increased in synovial membrane tissues from patients with OA (Smith et al, 1997). These studies, together with our results, suggest that expression of mPGES-1 in chondrocytes from patients with OA may be promoted by endogenous IL-1β and TNF-α released from cartilage and synovial membrane.

Functional coupling of mPGES-1 and COX-2

In almost all systems studied, the induction of mPGES-1 expression by proinflammatory stimuli was correlated with increased expression of COX-2 and PGE_2 generation. The coordinate up-regulation of COX-2 and mPGES-1 and attendant PGE_2 production are simultaneously reversed by glucocorticoids. In mPGES-1 knockout mice, LPS-stimulated production of PGE_2 by macrophages was blunted entirely, thus comfirming the absolute requirement of this enzyme for the COX-2-dependent delayed PGE_2-biosynthetic response. These studies suggest that a functional linkage exists between COX-2 and mPGES. To explore this, functional coupling of COX-2, but not COX-1, with mPGES has been demonstrated by co-transfection experiments performed in human embryonic kidney 293 cells. HEK 293 cells cotransfected with COX-2 and mPGES-1 produce higher amounts of PGE_2 then cells with either enzyme alone. An increase in PGE_2 production was also observed in cells cotransfected with mPGES and COX-1, their coupling became apparent only when a high concentration of AA was supplied exogenously (Murakami et al, 2000). This study provides the unequivocal evidence that PGE_2 generation by mPGES-1 occurs predominantly through the COX-2-dependent pathway, particularly during the delayed responses induced by proinflammatory stimulus. Moreover, specific COX-2 inhibitors reduce production of PGE_2 more then other stable prostaglandins in vivo. This observation suggests that the COX-2-dependent pathway may be more selectively linked to PGES than to the other

terminal PG synthases (Harada et al, 1998). Several reports have showed that COX-2 and mPGES-1 are colocalized in the perinuclear membrane. mPGES-1 and COX-2 showed perinuclear colocalization in IL-1β-stimulated RA synovial fibroblasts (Kojima et al, 2002) and chondrocyte (Kojima et al, 2004). These observations imply that colocalization of COX-2 and mPGES-1 in the same subcellular compartments critically affect their efficient coupling and might be related to the preferential increase of PGE_2 production after IL-1β stimulation due to functional linkage between these two enzymes.

Although COX-2 and mPGES-1 express very similarly in response to the same stimuli, there are differences in the specific timing for induction. Our time course of the expression of mPGES-1 in IL-1β stimulated chondrocytes analysis showed that mPGES-1 protein expression started to increase 6h post-stimulation with IL-1β, reached maximum at 24h, and remained elevated even 48h after IL-1β stimulation. This is in contrast to IL-1β- induced COX-2 in chondrocytes, in which expression is rapidly induced 2-3h, reaching a maximum at 8h, and then gradually decreases to reach basal levels at 24-36h. Expression of mPGES-1 was delayed compared with that of COX-2. In contrast, the level of cPGES protein was not affected by IL-1β. The promoter of the mPGES-1 gene lacks many of the elements usually associated with cytokine-inducible genes. No binding site for NF-κB, CRE or E-box has been found in the mPGES-1 promoter, as seen in COX-2 induction. The IL-1β induced COX-2 expression seems to involve NF-κB, but mPGES-1 promoter does not contain any such NF-κB site. Furthermore, the 3'region of mPGES lacks the AUUUA instability sequences found in the COX-2 gene (Forsberg et al, 2000). Thus, these findings suggest that the regulatory mechanisms for induction of the two enzymes mPGES-1 and COX-2 are different.

IL-1β inducted mPGES-1 expression at the transcriptional level

Among the various cytokines, we examined the effect of IL-1β, a key mediator in the pathogenesis of arthritis, on both the expression of mPGES-1 and the production of PGE_2 by OA chondrocytes. Our results showed that IL-1β induced mPGES-1 protein expression in a does-dependent manner. Our previous studies showed that mPGES-1

overexpression in OA synovial fibroblasts was stimulated with IL-1β (Cheng et al, 2004).

Upregulation of mPGES-1 expression by IL-1β occurred, at least in part, at the transcriptional level, as determined by real-time RT-PCR and transient transfection experiments. We analyzed the both effect of IL-1β on the expression of mPGES-1 mRNA and mPGES-1 promoter activity in articular chondrocytes from OA patients and our results showed that IL-1β induced a does-dependent increase in mPGES-1 mRNA expression and of mPGES-1 promoter activity. In contrast, IL-1β had no effect on the cPGES mRNA and promoter activity. Evidence is emerging that mPGES-1 is an inducible enzyme, the expression of which is markedly increased in various cells and tissues following proinflammatory stimuli. Inducible genes contain particular nucleotide elements within their promoter regions that are responsible for regulated transcription. It is known that the human mPGES-1 promoter contains several potential transcription factor-binding sites, including 2 GC boxes, 2 Barbie boxes, and an ary hydrocarbon response element. The intracellular signalling pathways that lead to upregulation of mPGES-1 are still unclear. However, it was recently shown that mPGES-1 expression in response to various stimuli is regulated by a transcription factor Egr-1. The binding of Egr-1 to the proximal GC box in the mPGES-1 gene promoter is an essential event that directs the regulatory expression of mPGES-1 in several cell types including macrophages, osteoblastic cells and human colonocytes. This is consistent with our previous finding that Egr-1 is important for mPGES-1 transcription in human synovial fibroblasts (Cheng et al, 2004). In electrophoretic mobility shift and supershift assays for Egr-1 binding sites in the mPGES-1 promoter, we observed that IL-1β induced DNA-binding activity of Egr-1 in human synovial fibroblasts (Cheng et al, 2004). Although we have not investigated Egr-1, it is very likely that this transcription factor may also play an important role in induced mPGES-1 gene expression in chondrocytes. Similar to our previous experiments in synovial fibroblasts by using EMSA and supershift assays, we can determine whether IL-1β could induce binding of Egr-1 to the mPGES-1 promoter in chondrocytes.

In addition to Egr-1, the mPGES-1 promoter contains binding sites for transcription factors such as AP-1. Although the role of those elements in IL-1β-

induced mPGES-1 transcription is still unknown, we cannot exclude the possibility that these transcription factors may be also involved in the induction of mPGES-1 expression. Recent data suggest a regulatory role for Erk and p38 MAPK and phosphatidylcholine phospholipase C on mPGES-1. For instance, mPGES-1 is stimulated in human chondrocytes by the proinflammatory cytokine IL-1β via activation of both ERK-1/2 and p38 MAPK in an isoform-specific manner (Masuko-Hongo et al, 2004). Moreover, other studies indicate the possible role of NF-κB and NF-IL6 in induction of mPGES-1 expression. In one study, data showed that IL-1β induces mPGES-1 expression through the transcription factor NF-κB in A549 cells (Catley et al, 2003). On the other hand, in vivo study of mice indicated that LPS failed to induce mPGES-1 expression in macrophages from NF-IL6-deficient mice (Uematsu et al, 2002). However, the mPGES-1 promoter contains neither NF-κB nor NF-IL6 responsive elements, suggesting that these transcription factors may regulate mPGES-1 expression via mechanisms that do not involve their direct interaction with the mPGES-1 promoter. More complete characterization of the mechanism involved in the regulation of mPGES-1 needs further investigation.

IL-1β, TNF-α and IL-17 synergistically stimulate the expression of mPGES-1

Cytokines such as IL-1 and TNF-α have been shown to play a pivotal role in pathologies of arthritis. IL-1 and TNF-α promote ECM degradation through the induction of MMPs, such as collagenase and stromelysin. In addition, these inflammatory cytokines inhibit proteoglycan synthesis and induce inflammation-mediating enzymes like COX-2 and iNOS. Human articular chondrocytes stimulated with IL-1, TNF-α, and LPS produce high levels of prostaglandins and NO. These mediators are present at elevated levels in articular joint tissues. In addition to IL-1β and TNF-α, several other cytokines can be detected in arthritic joints, such as IL-17. IL-17 has been implicated in the pathogenesis of arthritis and is believed to induce their effects also through enhancing production of a number of inflammatory and catabolic factors. Since all these proinfalmmatory cytokines are potent inducers of COX-2 expression and PGE_2 production in human articular chondrocytes, in the present study we examined the effect of both cytokines TNF-α and IL-17 on mPGES-1 expression.

We found that TNF-α and IL-17, like IL-1β, also induced mPGES-1 expression and PGE_2 production in a does-dependent manner. At optimal concentrations, the effect of TNF-α or IL-17 on mPGES-1 expression and PGE_2 production was less potent than IL-1β. Our data indicated that IL-17, TNF-α as potential additional players in the cytokine networks involved in arthritis and play potential role in the induction of inflammatory responses in chondrocytes.

IL-17 shares many properties with IL-1β and TNF-α. The three cytokines activate the common transcription factor NF-κB in a variety of cell types. They all stimulate stromal cells such as dermal and synovial fibroblasts, endothelial cells and epithelial cells to secrete IL-6, IL-8 and PGE_2. They also effect osteoclasts and bone destruction. Moreover, cartilage degradation is largely dependent on IL-1β and TNF-α and recent in vivo study showed the capacity of IL-17 to replace the catabolic function of IL-1β in cartilage damage during experimental arthritis (Koenders et al, 2005); Interactions between these three cytokines further amplify these effects. IL-17 induces IL-1β and TNF-α production by human macrophages (Jovanovic et al, 1998). More importantly, combination of IL-1β with TNF-α and IL-17 often leads to synergistic or additive effects, which further increase their biological effects.

Combination of IL-1β or TNF-α with IL-17 was found to be synergistic in bone stromal cells, in synovial fibroblasts, and in bone and meniscus explants. Many studies have considered these cytokine acting alone at concentrations, which may not be achieved, in vivo. In diseases such as RA, cytokines are present in combination but probably at lower concentrations. Since IL-1β, TNF-α, and IL-17 have many additive and/or synergistic effects in vitro and in vivo, in present study we extend these findings by examining the effect of different combinations of these cytokines on mPGES-1 expression and PGE_2 production. Our results showed that at a lower concentration, IL-1β, IL-17, and TNF-α alone had little or no effect on mPGES-1 expression and PGE_2 production. The combination of IL-1β, IL-17, and TNF-α were synergistic on induction of mPGES-1 protein expression. The combination of either of IL-1β and TNF-α or IL-1β and IL-17 resulted in a greater effect then IL-17 and TNF-α. Moreover, the effect of the combination of three cytokines was stronger than that of each of the two cytokines, suggesting that the combination of cytokines may be of importance in increased

expression of mPGES-1 and PGE_2 production. In contrast, the level of cPGES and mPGES-2 expression were not altered by these treatments. Since IL-1β, IL-17, and TNF-α are key mediators in arthritis, their control represents a major therapeutic goal. Our study demonstrated the potency of cytokine combination at low concentrations on mPGES-1 expression and PGE_2 production in OA chondrocytes. Accordingly, the understanding of synergistic activation is critical as this may lead to new therapeutic applications. Such mechanisms may need further explanation at transcriptional level.

PPARγ and 15d-PGJ_2 in inflammation and arthritis:

The biological effects of 15d-PGJ_2 relate to activation of PPARγ. PPARγ plays a role in articular joint cells such as articular chondrocytes, synovial fibroblast cells and osteoclasts. The consequences of PPARγ activation are complex, but evidence to date suggests that these transcriptional regulators have the anti-inflammatory ability to modulate inflammatory responses in vitro and in vivo.

Cartilage chondrocytes: Previous studies on PPAR expression patterns have shown that PPARγ is expressed in human articular cartilage and cultured chondrocytes. It is believed that MMPs and NO play a central role in articular inflammation and cartilage damage. 15d-PGJ_2 and other PPARγ activators, but not PPARα ligands inhibit IL-1β-induced NOS expression and synthesis and inhibit IL-1β-induced MMP-13 expression and production by these cells. The inhibitory effect of PPARγ activation is not restricted to IL-1β since TNF-α and IL-17 induced NO and MMP-13 production were also inhibited by 15d-PGJ_2 in the same cells (Fahmi et al, 2001). PPARγ ligands also inhibite the induced NOS expression in chondrocyte from OA patients. In addition to inhibition of MMP and NO production, the PPARγ activator 15d-PGJ_2 inhibit IL-1β inducted PGE_2 production and COX-2 expression by human chondrocytes (Fahmi et al, 2002) and 15d- PGJ_2 and troglitazone counteracted the IL-1β induced decrease in proteoglycan synthesis in rat chondrocytes. The inhibition occurs at least at the transcriptional level through a PPARγ-dependent pathway, probably by interfering with the activation of AP-1 and NF-κB. All these evidences indicated the protective effects of PPARγ activators in articular cartilage. In contrasted, 15d-PGJ_2 induced apoptosis of chondrocyte from OA or RA patients in a PPARγ-dependent manner and inhibition of

NF-κB and activation of P38 MAPK were found to be involved in 15d- PGJ_2 induced chondrocyte apoptosis. These results suggest that 15d- PGJ_2 may play a role in the pathogenesis of arthritic joint destruction via a regulation of chondrocyte apoptosis (Shan et al, 2004). Thus, 15d-PGJ_2 may have both chondroprotective and chondrodestructive effects.

Synovial fibroblast cells: PPARγ is expressed and transcriptionally functional in human synovial fibroblasts. Synovial fibroblast cells from patients with RA and OA also were shown to express PPARγ (Kawahito et al, 2000). PPARγ activators 15d-PGJ_2 inhibited IL-1β-induced MMP-1 synthesis in a dose-dependent manner in human synovial fibroblasts. The inhibitory effect of 15d-PGJ_2 occurs at the transcriptional level, at least in part, through inhibition of the transcription factors AP-1 activity. Similarly to chondrocytes, PPARγ activator 15d-PGJ_2 inhibited induced PGE_2 production and COX-2 expression by human synovial fibroblasts (Fahmi et al, 2002). Moreover, our previous study showed that both natural (15d-PGJ_2) and synthetic PPARγ ligands (troglitazone) inhibit IL-1β-induced mPGES-1 expression in human synovial fibroblasts. We demonstrated that this suppressive effect is transcriptional and PPARγ-dependent and the PPARγ activation suppresses mPGES-1 expression via negative interference with transcription factor Egr-1. Taken together, our results reveal a novel function of PPARγ, further supporting its role in the control of inflammatory responses (Cheng et al, 2004).

Accumulating data above have shown that PPARγ ligands inhibit expression of several genes involved in the pathogenesis of arthritis and suggest a possible role for 15d-PGJ_2 as well as PPARγ in regulation of human arthritis. Much attention has been focused on 15d-PGJ_2 because it is a high affinity ligand for PPARγ and because of its potent anti-inflammatory activity. In our present study, we assessed the effect of these molecules on mPGES-1 expression in chondrocytes. Our results showed that the PPARγ ligands 15d-PGJ_2 and troglitazone, but not the PPARα ligand Wy14643, repressed IL-1β-induced mPGES-1 expression. These data suggest that 15d-PGJ_2 prevented IL-1β-inducted mPGES-1 expression, at least in part, through a PPARγ-dependent mechanism.

Because arthritis is a complex process involving interactions of many cell types and mediators, it is difficult to extrapolate the above in vitro data to predict the in vivo

impact of PPARγ and its ligand 15d-PGJ_2. Animal model and human studies are essential to clarify the biological role of PPARγ and 15d-PGJ_2 in arthritis.

Intraperitoneal administration of PPARγ ligands (15d-PGJ_2 and troglitazone) ameliorated adjuvant-induced arthritis with suppression of pannus formation and mononuclear cell infiltration in female Lewis rats. Anti-inflammatory effects of 15d-PGJ_2 were more potent than troglitazone. These findings suggest that PPARγ may be an important immunoinflammatory mediator and its ligands, especially 15d-PGJ_2, may be useful in the treatment of RA (Kawahito et al, 2000). 15d-PGJ_2 reduced the expression of iNOS and COX-2 in the lungs of carrageenan-treated mice and in the joints from collagen-treated mice. Thus, 15d-PGJ_2 reduces the development of acute and chronic inflammation (Cuzzocrea et al, 2002).

Human subjects with active psoriatic arthritis PsA treated with a PPARγ agonist pioglitazone for 12 weeks showed improvement in the Psoriasis Area and Severity Index (PASI) in patients with more than 2% skin involvement and significant reduction of median tender joint count (interquartile range) and the median swollen joint count. Treatment with a PPARγ agonist appears to be a promising therapeutic principle in PsA (Bongartz et al, 2005).

PGE_2 functions as a positive feedback regulator of mPGES-1

In our present study, we evaluated the role of PGE_2, the end product of mPGES-1, in the repressing effect of PPARγ ligands. Our results showed that PGE_2 dose-dependently alleviated the suppressive effect of 15d-PGJ_2 or troglitazone on IL-1β - induced mPGES-1 expression and PGE_2 had no significant effect on unstimulated mPGES-1 expression suggesting that additional signals are provided by IL-1β stimulation, which PGE_2 alone cannot provide. This suggestion is supported by findings that inhibition of IL-1β-induced mPGES-1 expression by NSAID is restored by addition of PGE_2 (Kojima et al, 2003). PGE_2 exerts various physiological functions through the EP receptor, which has four subtypes (EP_1-EP_4). The EP_2 and EP_4 receptors increase cyclic AMP via activation of adenylate cyclase. EP_2 and EP_4 receptors are expressed in synovial fibroblasts from RA patients and in chondrocytes, making these cells likely targets for receptor-dependent action of PGE_2. Furthermore, excessive

production of PGE_2 has been detected in serum and synovial fluids of RA and OA patients. PGE_2 enhances the expression of mPGES-1 in synovial fibroblasts and chondrocytes may be by increasing cAMP through activation of EP_2 and EP_4 receptors. Thus, PGE_2 is a strong enhancer of IL-1β-induced mPGES-1 expression in synovial fibroblasts and chondrocytes. Autoregulation of mPGES-1 expression by PGE_2 may play an important role in the circle of inflammation associated with arthritis.

mPGES-1 as a novel drug target for arthritis

PGE_2 is a potent mediator of pain and inflammation, and high levels of this lipid mediator are observed in numerous disease states. Overproduction of PGE_2 clearly plays a central role in the pathogenesis of arthritis. The inhibition of PGE_2 production to control pain and to treat diseases such as RA to date has depended on nonsteroidal antiinflammatory agents such as aspirin. Clinicially, NSAIDs (COX inhibitors) have been used most frequently as prophylactic and therapeutic drugs for arthritis. Thus, agents that inhibit PGES enzymes could be also effective on the therapy of these diseases.

It has been shown that NSAIDs are widely used in Northern America and are used most commonly in the treatment of arthritis. Because of their anti-inflammatory and analgesic properties, NSAIDs are usually prescribed for the initial and long-term treatment of RA and they are also effective in controlling pain in patients with mild-to moderate OA. NSAIDs also are used to treat many other conditions such as headache, fever and gout.

With increased understanding of the biosynthesis of PGs and the demonstration in earlier work that the primary mechanism of NSAIDs in the treatment of inflammation is the inhibition of COX, which exists in two forms, leading to the inhibition of PG production. COX-1 appears to regulate many normal physiologic functions, and COX-2 mediates the inflammatory response. Inhibition of COX accounts for many of the side effects of NSAIDs, because NSAIDs inhibit PGI_2 and TXA_2 production in gastric mucosa, endothelial cells, the kidneys, and platelets. Despite their widespread use, NSAIDs are often associated with severe adverse effects, the most common being gastrointestinal (GI) toxicity.

It is believed that the anti-inflammatory actions of NSAIDs are primarily due to inhibition of COX-2 and that NSAID-induced adverse effects are largely caused by inhibition of COX-1. The identification of COX-2 as a key enzyme in PGE_2 synthesis resulted in the introduction of several COX-2 selective inhibitors. Theoretically, the new class of NSAIDs that inhibits COX-2 selectively should decrease inflammation but not influence normal physiologic functions and thus should cause fewer gastrointestinal side effects. Clinical data on the selective COX-2 inhibitors suggest that these agents may be as effective as traditional NSAIDs but are less likely to cause GI complications. Although these drugs have decreased gastrointestinal toxicity compared with traditional NSAIDs, they still have other unwanted side effects such as renal toxicity, thrombosis and increased risk of cardiovascular events. Therefore, a specific mPGES-1 inhibitor is desirable.

The inhibition of PGE_2 production to control pain and to treat arthritis to date has depended on NSAIDs. However, these agents inhibit the synthesis of all prostanoids. To produce biologically active PGE_2, terminal enzyme PGE synthases act downstream of COX-2 to catalyze the isomerization of COX-2 into PGE_2. Thus specific terminal enzyme mPGES-1 inhibitor should more specifically inhibit PGE_2 synthesis, while maintaining other normal physiologic prostanoid levels in the kidneys, brain and elsewhere.

Advantage with specific inhibition of mPGES-1 was also seen in mPGES-1-deficient mice. These animals displayed no abnormalities compared with wild-type controls, suggesting that other PGES substitute for mPGES-1 in normal physiology (Trebino et al, 2003). Since 15d-PGJ_2 is an inhibitor of mPGES-1 and has anti-inflammatory properties, studies with 15d-PGJ_2 and other specific inhibitors of mPGES-1 on various systems are needed to further elucidate its therapeutic potential.

D. CONCLUSION

Our data show that expression of mPGES-1 is upgulated in OA cartilage. The proinflammatory cytokines IL-1β, TNF-α, and IL-17 may be responsible for this up-regulation. Combined with results from previous studies showing a critical role of mPGES-1 in the synthesis of PGE_2 and the pathogenesis of arthritis, these data suggest that mPGE-1 constitutes a novel therapeutic target in the treatment of arthritis and possibly other diseases in which increased production of PGE_2 is implicated.

E. REFERENCE

Alaaeddine N, Di Battista JA, Pelletier JP, Kiansa K, Cloutier JM, Martel-Pelletier J. (1999) Differential effects of IL-8, LIF (pro-inflammatory) and IL-11 (anti-inflammatory) on TNF-alpha-induced PGE(2) release and on signalling pathways in human OA synovial fibroblasts. Cytokine.11(12):1020-30.

Amin AR, Attur M, Patel RN, Thakker GD, Marshall PJ, Rediske J, Stuchin SA, Patel IR, Abramson SB (1997) Superinduction of cyclooxygenase-2 activity in human osteoarthritis-affected cartilage. Influence of nitric oxide. J Clin Invest. 99(6):1231-7.

Amin AR.(1999) Regulation of tumor necrosis factor-alpha and tumor necrosis factor converting enzyme in human osteoarthritis. Osteoarthritis Cartilage. 7(4):392-4.

Anderson GD, Hauser SD, McGarity KL, Bremer ME, Isakson PC, Gregory SA (1996) Selective inhibition of cyclooxygenase (COX)-2 reverses inflammation and expression of COX-2 and interleukin 6 in rat adjuvant arthritis. J Clin Invest. 97(11):2672-9.

Berenbaum F, Jacques C, Thomas G, Corvol MT, Bereziat G, Masliah J. (1996) Synergistic effect of interleukin-1 beta and tumor necrosis factor alpha on PGE2 production by articular chondrocytes does not involve PLA2 stimulation. Exp Cell Res. 222(2):379-84.

Billinghurst RC, Dahlberg L, Ionescu M, Reiner A, Bourne R, Rorabeck C, Mitchell P, Hambor J, Diekmann O, Tschesche H, Chen J, Van Wart H, Poole AR (1997). Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. J Clin Invest 99:1534–1545

Bongartz T, Coras B, Vogt T, Scholmerich J, Muller-Ladner U. (2005) Treatment of active psoriatic arthritis with the PPARgamma ligand pioglitazone: an open-label pilot study. Rheumatology (Oxford). 44(1):126-9.

Braissant O, Foufelle F, Scotto C, Dauca M, Wahli W.(1996) Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. Endocrinology. 137(1):354-66

Brandt KD; Doherty M; and Lohmander LS (2003) Osteoarthritis 2nd edition P1-189

Brater DC, Harris C, Redfern JS, Gertz BJ. (2001) Renal effects of COX-2-selective inhibitors. Am J Nephrol. 21(1):1-15

Buckwalter JA, Lane LE.(1997) Athletics and osteoarthritis. Am J Sports Med. 25: 873-81

Catley MC, Chivers JE, Cambridge LM, Holden N, Slater DM, Staples KJ, Bergmann MW, Loser P, Barnes PJ, Newton R. (2003) IL-1beta-dependent activation of NF-kappaB mediates PGE2 release via the expression of cyclooxygenase-2 and microsomal prostaglandin E synthase. FEBS Lett. 547(1-3): 75-9.

Cheng S, Afif H, Martel-Pelletier J, Pelletier JP, Li X, Farrajota K, Lavigne M, Fahmi H. (2004) Activation of peroxisome proliferator-activated receptor γ inhibits interleukin-1β-induced membrane-associated prostaglandin E_2 synthase-1 expression in human synovial fibroblasts by interfering with Egr-1. J Biol Chem. 21;279(21):22057-65.

Claveau D, Sirinyan M, Guay J, Gordon R, Chan CC, Bureau Y, Riendeau D, Mancini JA. (2003) Microsomal prostaglandin E synthase-1 is a major terminal synthase that is selectively up-regulated during cyclooxygenase-2-dependent prostaglandin E_2 production in the rat adjuvant-induced arthritis model. The Journal of Immunology. 170(9):4738-44.

Corton JC, Anderson SP, Stauber A.(2000) Central role of peroxisome proliferator-activated receptors in the actions of peroxisome proliferators. Annu Rev Pharmacol Toxicol 40:491-518.

Crofford LJ, Tan B, Mccarthy CJ, Hla T (1997) Involvement of nuclear factor kappa B in the regulation of cyclooxygenase-2 expression by interleukin-1 in rheumatoid synoviocytes. Arthritis Rheum 40:226-36

Crofford L.J. (1997) COX-1 and COX-2 tissue expression: implications and predictions. J Rheumatol Suppl. 49:15-9.

Crofford LJ. (2002) Specific cyclooxygenase-2 inhibitors: what have we learned since they came into widespread clinical use? Curr Opin Rheumatol 14:225-30.

Cuzzocrea S, Wayman NS, Mazzon E, Dugo L, Di Paola R, Serraino I, Britti D, Chatterjee PK, Caputi AP, Thiemermann C. (2002) The cyclopentenone prostaglandin 15-deoxy-Delta(12,14)-prostaglandin J(2) attenuates the development of acute and chronic inflammation. Mol Pharmacol. 61(5):997-1007.

Dayer JM, Zavadil-Grob C, Ucla C, Mach B. (1984) Induction of human interleukin 1 mRNA measured by collagenase- and prostaglandin E2-stimulating activity in rheumatoid synovial cells. Eur J Immunol. 14(10):898-901.

Desvergene B, Wahli W (1999) Peroxisome proliferator-activated receptor: Nuclear control of metabolism. Endocr Rev 20:649-688

Dingle JT, Saklatvala J, Hembry R, Tyler J, Fell HB, Jubb R. (1979) A cartilage catabolic factor from synovium. Biochem J. 184(1):177-80.

Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, Lipsky PE (1998). Cyclooxygenase in biology and disease. FASEB Journal 12: 1063-1073

Duffy DM, Seachord CL, Dozier BL. (2005) Microsomal prostaglandin E synthase-1 (mPGES-1) is the primary form of PGES expressed by the primate periovulatory follicle. Hum Reprod. 20(6):1485-92.

Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. (1994) Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology. 107(4):1183-8.

Engblom D, Saha S, Engstrom L, Westman M, Audoly LP, Jakobsson PJ, Blomqvist A. (2003) Microsomal prostaglandin E synthase-1 is the central switch during immune-induced pyresis. Nat Neurosci. 6(11):1137-8.

Escher P, Wahli W. (2000) Peroxisome proliferator-activated receptors: insight into multiple cellular functions. Mutat Res 448:121-38.

Evensen S, Spigset O, Slordal L. (2005) COX-2 inhibitors--one step forward and two steps back. Tidsskr Nor Laegeforen. 125(7):875-8.

Fahmi H, Di Battista JA, Pelletier JP, Mineau F, Ranger P, Martel-Pelletier J. (2001) Peroxisome proliferator--activated receptor gamma activators inhibit interleukin-1beta-induced nitric oxide and matrix metalloproteinase 13 production in human chondrocytes. Arthritis Rheum. 44(3):595-607.

Fahmi H, Pelletier JP, Martel-Pelletier J. (2002) PPARgamma ligands as modulators of inflammatory and catabolic responses on arthritis. An overview. J Rheumatol. 29(1):3-14.

Faour W, He Y, de Ladurantaye M, He W, Quintero M, Mancini A. (2001) Prostaglandin E2 regulates the level and stability of cyclooxygenase-2 mRNA through activation of p38 mitogen-activated protein kinase in interleukin-1 beta treated synovial fibroblasts. J Biol Chem 276: 31720-31

Felson DT (1990). The epidemiology of knee osteoarthritis: results from the framingham osteoarthritis study. Sem Arthritis Rheum 20: 42-50.

Felson DT, Zhang Y, Anthony JM, Naimark A, Anderson JJ. (1992) Weight loss reduce the risk for symptomatic knee osteoarthritis in women. The Framingham study. Ann Intern Med. 116: 535-539

Felson DT, Zhang Y, Hannan MT, Naimark A, Weissman BN, Aliabadi P, Levy D. (1995) The incidence and natural history of knee osteoarthritis in the elderly: the Framingham Osteoarthritis study. Arthritis Rheum 38: 1500-5

Felson DT, Lawrence RC, Dieppe PA, Hirsch R, Helmick CG, Jordan JM, Kington RS, Lane NE, Nevitt MC, Zhang Y, Sowers M, McAlindon T, Spector TD, Poole AR, Yanovski SZ, Ateshian G, Sharma L, Buckwalter JA, Brandt KD, Fries JF. (2000) Osteoarthritis: New Insights Part1: The disease and its risk factors Annals of Internal Medicine Volume 133. Number 8 p635-645

Filion F, Bouchard N, Goff AK, Lussier JG, Sirois J. (2001) Molecular Cloning and Induction of Bovine Prostaglandin E Synthase by Gonadotropins in Ovarian Follicles Prior to Ovulation in Vivo.J Biol Chem. 276(36):34323-30

Fitzpatrick FA, Wynalda MA. (1983) Albumin-catalyzed metabolism of prostaglandin D2. Identification of products formed in vitro. J Biol Chem. 258(19):11713-8.

Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM (1995) 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. Cell. 83(5):803-12

Forsberg L, Leeb L, Thoren S, Morgenstern R, Jakobsson P. (2000). Human glutathione dependent prostaglandin E synthase: gene structure and regulation FEBS Lett. 471(1):78-82.

Gallois C, Habib A, Tao J, Moulin S, Maclouf J, Mallat A, Lotersztajn S. (1998) Role of NF-kappaB in the antiproliferative effect of endothelin-1 and tumor nerosis factor-alpha in human hepatic stellate cells. Involvement of cyclooxygenase-2. J Biol Chem 273:23183-90

Geng Y, Valbracht J, Lotz M (1996) Selective activation of the mitogen-activated protein kinase subgroups c-Jun NH2 terminal kinase and p38 by IL-1 and TNF in human articular chondrocytes J Clin Invest 98: 2425-2430

Goldring MB. (1999) The role of cytokines as inflammatory mediators in osteoarthritis: lessons from animal models.Connect Tissue Res. 40(1):1-11. Review.

Han R, Tsui S, Smith TJ. (2002) Up-regulation of prostaglandin E2 synthesis by interleukin-1beta in human orbital fibroblasts involves coordinate induction of prostaglandin-endoperoxide H synthase-2 and glutathione-dependent prostaglandin E2 synthase expression.J Biol Chem. 277(19):16355-64.

Haq I, Murphy E, Dacre J. (2003) Osteoarthritis. Postgrad. Med. J. 79; 377-383

Harada Y, Kawamura M, Hatanaka K, Saito M, Ogino M, Ohno T, Ogino K, Yang Q. (1998) Differing profiles of prostaglandin formation inhibition between selective prostaglandin H synthase-2 inhibitors and conventional NSAIDs in inflammatory and non-inflammatory sites of the rat. Prostaglandins Other Lipid Mediat. 55(5-6):345-58.

Hashimoto S., Ochs R.L., Komiya S., Lotz M. (1998). Linkage of chondrocyte apoptosis and cartilage degradation in human osteoarthritis. Arthritis Rheum 41: 1632-8

Hunter DJ, March L, Sambrook PN (2002) Knee osteoarthritis: the influence of environmental factors. Clin Exp Rheumatol 20;93-100.

Ijpenberg A, Jeannin E, Wahli W, Desvergne B. (1997) Polarity and specific sequence requirements of peroxisome proliferator-activated receptor (PPAR)/retinoid X receptor heterodimer binding to DNA. A functional analysis of the malic enzyme gene PPAR response element. J Biol Chem. 272(32):20108-17.

Irvine RF.(1982) How is the level of free arachidonic acid controlled in mammalian cells? Biochem J. 204(1):3-16.

Jakobsson PJ, Thoren S, Morgenstern R, Samuelsson B. (1999) Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proc Natl Acad Sci USA 96:7220–5.

Jakobsson PJ, Morgenstern R, Mancini J, Ford-Hutchinson A, Persson B. (2000) Membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG). A widespread protein superfamily. Am J Respir Crit Care Med.161(2 Pt 2):S20-4.

Jovanovic DV, DiBattista JA, Martel-Pelletier J, Jolicoeur FC, He Y, Zhang M, Mineau F, Pelletier JP. (1998) IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. J Immunol 160:3513-3521

Kamei D, Murakami M, Nakatani Y, Ishikawa Y, Ishii T, Kudo I. (2003) Potential role of microsomal prostaglandin E synthase-1 in tumorigenesis. J Biol Chem. 278(21):19396-405.

Kamei D, Yamakawa K, Takegoshi Y, Mikami-Nakanishi M, Nakatani Y, Oh-Ishi S, Yasui H, Azuma Y, Hirasawa N, Ohuchi K, Kawaguchi H, Ishikawa Y, Ishii T, Uematsu S, Akira S, Murakami M, Kudo I. (2004) Reduced pain hypersensitivity and

inflammation in mice lacking microsomal prostaglandin e synthase-1. J Biol Chem. 279(32):33684-95.

Kawahito Y, Kondo M, Tsubouchi Y, Hashiramoto A, Bishop-Bailey D, Inoue K, Kohno M, Yamada R, Hla T, Sano H. (2000) 15-deoxy-delta(12,14)-PGJ(2) induces synoviocyte apoptosis and suppresses adjuvant-induced arthritis in rats. J Clin Invest. 106(2):189-97.

Koenders MI, Lubberts E, Oppers-Walgreen B, van den Bersselaar L, Helsen MM, Kolls JK, Joosten LA, van den Berg WB. (2005) Induction of cartilage damage by overexpression of T cell interleukin-17A in experimental arthritis in mice deficient in interleukin-1. Arthritis Rheum. 52(3):975-83.

Kojima F, Naraba H, Sasaki Y, Okamoto R, Koshino T, Kawai S. (2002) Coexpression of microsomal prostaglandin E synthase with cyclooxygenase-2 in human rheumatoid synovial cells. J Rheumatol. 29(9):1836-42

Kojima F, Naraba H, Sasaki Y, Beppu M, Aoki H, Kawai S. (2003) Prostaglandin E2 is an enhancer of interleukin-1beta-induced expression of membrane-associated prostaglandin E synthase in rheumatoid synovial fibroblasts. Arthritis Rheum. 48(10):2819-28

Kojima F, Naraba H, Miyamoto S, Beppu M, Aoki H, Kawai S. (2004) Membrane-associated prostaglandin E synthase-1 is upregulated by proinflammatory cytokines in chondrocytes from patients with osteoarthritis. Arthritis Res Ther. 6(4):R355-65.

Koopman WJ et al (1997) Arthritis and allied condition A textbook of rheumatology 13th Edition p255-308

Kudo I, Murakami M. (2002) Phospholipase A2 enzymes. Prostaglandins Other Lipid Mediat. 68-69:3-58.

Lazarus M, Craig J. Munday, Naomi Eguchi, Shigeko Matsumoto, Gary J. Killian, Bruno Kilunga Kubata and Yoshihiro Urade (2002) Immunohistochemical Localization of Microsomal PGE Synthase-1 and Cyclooxygenases in Male Mouse Reproductive Organs Endocrinology. 143(6):2410-9

LeGrand A, Fermor B, Fink C, Pisetsky DS, Weinberg JB, Vail TP, Guilak F.(2001) Interleukin-1, tumor necrosis factor alpha, and interleukin-17 synergistically up-regulate nitric oxide and prostaglandin E2 production in explants of human osteoarthritic knee menisci. Arthritis Rheum. 44(9):2078-83

Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). J Biol Chem. 270(22):12953-6.

Lehmann JM, Lenhard JM, Oliver BB, Ringold GM, Kliewer SA. (1997) Peroxisome proliferator-activated receptors alpha and gamma are activated by indomethacin and other non-steroidal anti-inflammatory drugs. J Biol Chem 272(6):3406-10.

Li S, Wang Y, Matsumura K, Ballou LR, Morham SG, Blatteis CM (1999) The febrile response to lipopolysaccharide is blocked in cyclooxygenase-2(-/-), but not in cyclooxygenase-1(-/-) mice. Brain Res. 825(1-2):86-94.

Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM, Dey SK (1997) Multiple female reproductive failures in cyclooxygenase 2-deficient mice. Cell. 91(2):197-208

Lotz M (1999) The role of nitric oxide in articular cartilage damage. Rheum Dis Clin N Amer 25, 269-82

Lubberts E, Joosten LA, Oppers B, van den Bersselaar L, Coenen-de Roo CJ, Kolls JK, Schwarzenberger P, van de Loo FA, van den Berg WB. (2001) IL-1-independent role of IL-17 in synovial inflammation and joint destruction during collagen-induced arthritis. J Immunol. 167(2):1004-13

Malemud CJ (1999) Fundamental pathways in osteoarthritis: An overview . Frontiers in Bioscience 4, d659-661.

Mancini JA, Blood K, Guay J, Gordon R, Claveau D, Chan CC, Riendeau D (2001) Cloning, expression, and up-regulation of inducible rat prostaglandin e synthase during lipopolysaccharide-induced pyresis and adjuvant-induced arthritis. J Biol Chem 276(6):4469-75

Marnett LJ. (1992) Aspirin and the potential role of prostaglandins in colon cancer. Cancer Res. 52(20):5575-89

Martel-Pelletier J, McCollum R, DiBattista J, Faure MP, Chin JA, Fournier S, Sarfati M, Pelletier JP. (1992) The interleukin-1 receptor in normal and osteoarthritic human articular chondrocytes. Identification as the type I receptor and analysis of binding kinetics and biologic function. Arthritis Rheum. 35(5):530-40

Masuko-Hongo K, Berenbaum F, Humbert L, Salvat C, Goldring MB, Thirion S. (2004) Up-regulation of microsomal prostaglandin E synthase 1 in osteoarthritic human cartilage: critical roles of the ERK-1/2 and p38 signaling pathways. Arthritis Rheum. 50(9):2829-38.

Meade EA, McIntyre TM, Zimmerman GA, Prescott SM. (1999) Peroxisome proliferators enhance cyclooxygenase-2 expression in epithelial cells. J Biol Chem. 274(12):8328-34.

Melchiorri C, Meliconi R, Frizziero L, Silvestri T, Pulsatelli L, Mazzetti I, Borzi RM, Uguccioni M, Facchini A.(1998) Enhanced and coordinated in vivo expression of inflammatory cytokines and nitric oxide synthase by chondrocytes from patients with osteoarthritis. Arthritis Rheum.41(12):2165-74.

Merlie JP, Fagan D,MuddJ, Needleman P. (1988.) Isolation and characterization of the complementary DNA for sheep seminal vesicle prostaglandin endoperoxide synthase (cyclooxygenase). J. Biol. Chem. 263(8):3550-3

Monneret G, Cossette C, Gravel S, Rokach J, Powell WS. (2003) 15R-methyl-prostaglandin D2 is a potent and selective CRTH2/DP2 receptor agonist in human eosinophils. J Pharmacol Exp Ther. 304(1):349-55.

Morham SG, Langenbach R, Loftin CD, Tiano HF, Vouloumanos N, Jennette JC, Mahler JF, Kluckman KD, Ledford A, Lee CA, Smithies O. (1995) Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. Cell. 83(3):473-82

Morris JK, Richards JS.(1996) An E-box region within the prostaglandin endoperoxide synthase-2 (PGS-2) promoter is required for transcription in rat ovarian granulosa cells. J Biol Chem. 271(28):16633-43.

Mukherjee R, Jow L, Noonan D, McDonnell DP (1994) Human and rat peroxisome proliferator activated receptors (PPARs) demonstrate similar tissue distribution but different responsiveness to PPAR activators. J Steroid Biochem Mol Biol. 51 (3-4): 157-66.

Murakami M, Naraba H, Tanioka T, Semmyo N, Nakatani Y, Kojima F, Ikeda T, Fueki M, Ueno A, Oh S, Kudo I. (2000) Regulation of prostaglandin E2 biosynthesis by inducible membrane-associated prostaglandin E2 synthase that acts in concert with cyclooxygenase-2. J Biol Chem 275(42):32783-92

Murakami M, Nakashima K, Kamei D, Masuda S, Ishikawa Y, Ishii T, Ohmiya Y, Watanabe K, Kudo I. (2003) Cellular prostaglandin E2 production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenases-1 and -2. J Biol Chem. 278 (39): 37937-47.

Murray CJL, Lopez AD. (1996) The global burden of disease. Geneva: World Health Organisation

Myers LK, Kang AH, Postlethwaite AE, Rosloniec EF, Morham SG, Shlopov BV, Goorha S, Ballou LR. (2000) The genetic ablation of cyclooxygenase 2 prevents the development of autoimmune arthritis. Arthritis Rheum. 43(12):2687-93

Naraba H, Yokoyama C, Tago N, Murakami M, Kudo I, Fueki M, Oh-Ishi S, Tanabe T. (2002) Transcriptional regulation of the membrane-associated prostaglandin E2 synthase gene. Essential role of the transcription factor Egr-1. J Biol Chem. 277(32): 28601-8.

Narumiya S (1995) Structures, properties and distribution of prostanoid receptors. Adv Prostaglandin Trombox leukotriene Res 23:17-22

Nevitt, MC et al (1994) Current use of oral estrogen is associated with a decreased prevalence of radiographic hip OA in elderly white women. Arthritis Rheum 37(suppl.): S212

Newton R, Seybold J, Kuitert LM, Bergmann M, Barnes PJ. (1998) Repression of cyclooxygenase-2 and prostaglandin E2 release by dexamethasone occurs by transcriptional and post-transcriptional mechanisms involving loss of polyadenylated mRNA. J Biol Chem. 273(48): 32312-21.

Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF, Taketo MM. (1996) Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell. 87(5):803-9.

Page Thomas DP, King B, Stephens T, Dingle JT.(1991) In vivo studies of cartilage regeneration after damage induced by catabolin/interleukin-1. Ann Rheum Dis. 50(2):75-80

Petrova TV, Akama KT, Van Eldik LJ. (1999) Cyclopentenone prostaglandins suppress activation of microglia: down-regulation of inducible nitric-oxide synthase by 15-deoxy-Delta12,14-prostaglandin J2. Proc Natl Acad Sci U S A. 96(8):4668-73.

Pettipher ER, Higgs GA, Henderson B. (1986) Interleukin 1 induces leukocyte infiltration and cartilage proteoglycan degradation in the synovial joint. Proc Natl Acad Sci U S A. 83(22): 8749-53

Picot D, Loll PJ, Garavito RM (1994) The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1. Nature 367: 243-249

Pietila P, Moilanen E, Seppala E, Nissila M, Lepisto P, Laitinen O, Vapaatalo H.Differences in the production of arachidonic acid metabolites between healthy and rheumatic synovial fibroblasts in vitro. (1984) A preliminary study. Scand J Rheumatol. 13(3):243-6.

Pilbeam CC, Harrison JR and Raisz LG. (1996) Prostaglandins and bone metabolism. In principles of Bone Biology (Bilezikian J, Raisz L and Rodan G., eds), pp715-728, Academic Press, New York

Poole AR (1999) An introduction to the pathophysiology of osteoarthritis. Frontiers in Bioscience 4:D662-70

Poole A.R, Kojima T, Yasuda T, Mwale F, Kobayashi M., and Laverty S. (2001) CARTILAGE BIOLOGY: Composition and Structure of Articular Cartilage. Clinical Orthopaedics and Related Research No. 391S, pp. S26–S33

Poole AR, Kobayashi M, Yasuda T, Laverty S, Mwale F, Kojima T, Sakai T, Wahl C, El-Maadawy S, Webb G, Tchetina E, Wu W (2002) Type II collagen degradation and its regulation in articular cartilage in osteoarthritis. Ann. Rheum. Dis 61:p78-81

Portanova JP, Zhang Y, Anderson GD, Hauser SD, Masferrer JL, Seibert K, Gregory SA, Isakson PC. (1996) Selective neutralization of prostaglandin E2 blocks inflammation, hyperalgesia, and interleukin 6 production in vivo. J Exp Med 184:883-91

Quraishi O, Mancini JA, Riendeau D (2002) Inhibition of inducible prostaglandin E(2) synthase by 15-deoxy-Delta(12,14)-prostaglandin J(2) and polyunsaturated fatty acids. Biochem Pharmacol. 63(6):1183-9

Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. (1998) The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature. 391(6662):79-82.

Saegusa M, Murakami M, Nakatani Y, Yamakawa K, Katagiri M, Matsuda K, Nakamura K, Kudo I, Kawaguchi H. (2003) Contribution of membrane-associated prostaglandin E2 synthase to bone resorption. J Cell Physiol. 197(3):348-56

Saklatvala J, Pilsworth LM, Sarsfield SJ, Gavrilovic J, Heath JK. (1984) .Pig catabolin is a form of interleukin 1. Cartilage and bone resorb, fibroblasts make prostaglandin and collagenase, and thymocyte proliferation is augmented in response to one protein. Biochem J. 224(2):461-6

Sakuma Y, Tanaka K, Suda M, Komatsu Y, Yasoda A, Miura M, Ozasa A, Narumiya S, Sugimoto Y, Ichikawa A, Ushikubi F, Nakao K. (2000) Impaired bone resorption by

lipopolysaccharide in vivo in mice deficient in the prostaglandin E receptor EP4 subtype. Infect Immun. 68(12):6819-25.

Samad TA, Moore KA, Sapirstein A, Billet S, Allchorne A, Poole S, Bonventre JV, Woolf CJ. (2001) Interleukin-1beta-mediated induction of COX-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature 410: 471-5

Satoh K, Nagano Y, Shimomura C, Suzuki N, Saeki Y, Yokota H (2000) Expression of prostaglandin E synthase mRNA is induced in ß-amyloid treated rat astrocytes. Neurosci Lett 283:221–223

Scheinman RI, Cogswell PC, Lofquist AK, Baldwin Jr AS. (1995) Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. Science. 270(5234):283-6

Shan ZZ, Masuko-Hongo K, Dai SM, Nakamura H, Kato T, Nishioka K. (2004) A potential role of 15-deoxy-delta(12,14)-prostaglandin J2 for induction of human articular chondrocyte apoptosis in arthritis. J Biol Chem. 279(36):37939-50.

Sheng H, Williams CS, Shao J, Liang P, DuBois RN, Beauchamp RD. (1998) Induction of cyclooxygenase-2 by activated Ha-ras oncogene in Rat-1 fibroblasts and the role of mitogen-activated protein kinase pathway. J. Biol. Chem. 273:22120–27

Shlopov BV, Lie WR, Mainardi CL, Cole AA, Chubinskaya S, Hasty KA (1997): Osteoarthritic lesions. Involvement of three different collagenases. Arthritis Rheum 40, 2065-74

Shureiqi I, Jiang W, Zuo X, Wu Y, Stimmel JB, Leesnitzer LM, Morris JS, Fan HZ, Fischer SM, Lippman SM. (2003) The 15-lipoxygenase-1 product 13-S-hydroxyoctadecadienoic acid down-regulates PPAR-delta to induce apoptosis in colorectal cancer cells. Proc Natl Acad Sci U S A. 100(17):9968-73.

Siegle I, Klein T, Backman JT, Saal JG, Nusing RM, Fritz P (1998) Expression of cyclooxygenase-1 and cyclooxygenase-2 in human synovial tissue: Differential elevation of cyclooxygenase-2 in inflammatory joint diseases. Arthritis Rheum 41:122-9.

Smith MD, Triantafillou S, Parker A, Youssef PP, Coleman M. (1997) Synovial membrane inflammation and cytokine production in patients with early osteoarthritis. J Rheumatol 24: 365-371

Smith WL, DeWitt DL, Garavito RM (2000) CYCLOOXYGENASES: Structural, Cellular, and Molecular Biology Annu. Rev. Biochem. 69:145–82

Soler M, Camacho M, Escudero JR, Iniguez MA, Vila L (2000) Human vascular smooth muscle cells but not endothelial cells express prostaglandin E synthase. Circ Res 87:504–507

Spencer AG, Thuresson E, Otto JC, Song I, Smith T, DeWitt DL, Garavito RM, Smith WL. (1999) The membrane binding domains of prostaglandin endoperoxide H synthases 1 and 2. Peptide mapping and mutational analysis. J. Biol. Chem. 274(46):32936-42

Stichtenoth DO, Thoren S, Bian H, Peters-Golden M, Jakobsson PJ, Crofford LJ (2001). Microsomal prostaglandin E synthase is regulated by proinflammatory

cytokines and glucocorticoids in primary rheumatoid synovial cells. J Immunol. 167(1):469-74.

Stockwell RA, Meachin G: (1979) The Chondrocytes. In Freeman MAR (ed). Adult Articular Cartilage. Ed 2.Tunbridge Wells, United Kingdom, Pitman Medical 69–144

Straus DS, Pascual G, Li M, Welch JS, Ricote M, Hsiang CH, Sengchanthalangsy LL, Ghosh G, Glass CK (2000) 15-deoxy-delta 12,14-prostaglandin J2 inhibits multiple steps in the NF-kappa B signaling pathway. Proc Natl Acad Sci U S A. 97(9):4844-9

Studer RK, Levicoff E, Georgescu H, Miller L, Jaffurs D and Evans CH (2000) Nitric oxide inhibits chondrocyte response to IGF-1: inhibition of IGF-IRβ tyrosine phosphorylation. Am J Physiol Cell Physiol 279: 961–969

Subbaramaiah K, Telang N, Ramonetti JT, Araki R, DeVito B, Weksler BB, Dannenberg AJ. (1996) Transcription of cyclooxygenase-2 is enhanced in transformed mammary epithelial cells. Cancer Res 56, 4424-4429

Tanaka K, Hirai H, Takano S, Nakamura M, Nagata K. (2004) Effects of prostaglandin D2 on helper T cell functions. Biochem Biophys Res Commun. 316(4):1009-14

Tanabe T, Tohnai N. (2002) Cyclooxygenase isozymes and their gene structures and expression. Prostaglandins other lipid Mediat 68-69: 95-114.

Tanioka T, Nakatani Y, Semmyo N, Murakami M, Kudo I. (2000) Molecular identification of cytosolic prostaglandin E2 synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E2 biosynthesis. J Biol Chem. 275(42):32775-82.

Thomas B, Berenbaum F, Humbert L,Bian H, Bereziat G, Crofford L, Olivier JL (2000) Critical role of C/EBPdelta and C/EBPbeta factors in the stimulation of the cyclooxygenase-2 gene transcription by interleukin-1beta in articular chondrocytes. Eur J Biochem. 267(23):6798-809.

Thomas B, Thirion S, Humbert L, Tan L, Goldring MB, Bereziat G, Berenbaum F. (2002). Differentiation regulated interleukin-1 beta-induced cyclooxygenase-2 in human articular chondrocytes: role of p38 mitogen-activated protein kinase. Biochem J 362: 367-373

Tilley SL, Audoly LP, Hicks EH, Kim HS, Flannery PJ, Coffman TM, Koller BH (1999) Reproductive failure and reduced blood pressure in mice lacking the EP2 prostaglandin E_2 receptor. J Clin Invest. 103(11):1539-45.

Trebino CE, Stock JL, Gibbons CP, Naiman BM, Wachtmann TS, Umland JP, Pandher K, Lapointe JM, Saha S, Roach ML, Carter D, Thomas NA, Durtschi BA, McNeish JD, Hambor JE, Jakobsson PJ, Carty TJ, Perez JR, Audoly LP (2003) Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. Proc Natl Acad Sci U S A. 100(15):9044-9.

Tsafriri A (1995) Ovulation as a tissue remodelling process. Proteolysis and cumulus expansion. Adv Exp Med Biol.377:121-40.

Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN. (1998) Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell 93, 705-716

Uematsu S, Matsumoto M, Takeda K, Akira S. (2002) Lipopolysaccharide-dependent prostaglandin E(2) production is regulated by the glutathione-dependent prostaglandin E(2) synthase gene induced by the Toll-like receptor 4/MyD88/NF-IL6 pathway. J Immunol. 168(11):5811-6.

Van den Berg WB, Joosten LA, Kollias G, Van De Loo FA (1999) Role of tumour necrosis factor alpha in experimental arthritis: separate activity of interleukin 1beta in chronicity and cartilage destruction.Ann Rheum Dis. 58 Suppl 1:I40-8.

Wahli W, Braissant O, Desvergne B. (1995) Peroxisome proliferator activated receptors: transcriptional regulators of adipogenesis, lipid metabolism and more. Chem Biol. 2(5):261-6.

Watanabe K, Kurihara K, Suzuki T. (1999) Purification and characterization of membrane-bound prostaglandin E synthase from bovine heart. Biochim Biophys Acta.1439(3):406-14.

Webb GR, Westacott CI, Elson CJ (1997) Chondrocyte tumor necrosis factor receptors and focal loss of cartilage in osteoarthritis. Osteoarthritis Cartilage. 5(6):427-37.

Westman M, Korotkova M, af Klint E, Stark A, Audoly LP, Klareskog L, Ulfgren AK, Jakobsson PJ. (2004) Expression of microsomal prostaglandin E synthase 1 in rheumatoid arthritis synovium. Arthritis Rheum. 50(6):1774-80

Williams CS, Mann M, Dubois RN. (1999) The role of cyclooxygenase in inflammation, cancer, and development. Oncogene 18: 7908-7916

Wu JJ, Lark MW, Chun LE, Eyre DR (1991). Sites of stromelysin cleavage in collagen types II, IX, X, and XI of cartilage. J Biol Chem. 266(9):5625-8

Xie W, Herschman HR. (1995) v-src induces prostaglandin synthase 2 gene expression by activation of the c-Jun N-terminal kinase and the c-Jun transcription factor. J Bio Chem. 270(46):27622–28

Xu XM, Tang JL, Chen X, Wang LH, Wu KK. (1997) Involvement of two Sp1 elements in basal endothelial prostaglandin H synthase-1 promoter activity. J Biol Chem. 272(11):6943-50.

Yamagata K, Andreassson KI, Kaufman WE, Barnes CA, Worley PF. (1993) Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. Neuron. 11(2):371-86

Yamagata K, Matsumura K. (2001) Coexpression of Microsomal-Type Prostaglandin E Synthase with Cyclooxygenase-2 in Brain Endothelial Cells of Rats during Endotoxin-Induced Fever. The Journal of Neuroscience 21(8):2669-2677

Yamamoto K, Arakawa T, Ueda N, Yamamoto S (1995) Transcriptional roles of nuclear factor kappa B and nuclear factor-interleukin-6 in the tumor necrosis factor alpha-dependent induction of cyclooxygenase-2 in MC3T3-E1 cells. J Biol Chem. 270(52):31315-20

Yang-Yen HF, Chambard JC, Sun YL, Smeal T, Schmidt TJ, Drouin J, Karin M. (1990) Transcriptional interference between c-Jun and the glucocorticoid receptor:

mutual inhibition of DNA binding due to direct protein-protein interaction. Cell 62:1205-15

Zhang X, Young HA. (2002) PPAR and immune system--what do we know? Int Immunopharmacol. 2(8):1029-44

Zhang Y, Schneider A, Rao R, Lu WJ, Fan X, Davis L, Breyer RM, Breyer MD, Guan Y. (2003) Genomic structure and genitourinary expression of mouse cytosolic prostaglandin E(2) synthase gene. Biochim Biophys Acta. 1634(1-2):15-23

Zheng H, Fletcher D, Kozak W, Jiang M, Hofmann KJ, Conn CA, Soszynski D, Grabiec C, Trumbauer ME, Shaw A, et al. (1995) Resistance to fever induction and impaired acute-phase response in interleukin-1 beta-deficient mice. Immunity. 3(1):9-19.

Zubay G (1988) Biochemistry 2nd Edition p715-724