The Autotaxin-Lysophosphatidic acid pathway in pathogenesis of rheumatoid arthritis

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Abstract

Lysophosphatidic acid (LPA) is a phospholipid that is mainly produced by the hydrolysis of lysophosphatidylcholine (LPC) by lysophospholipase D, which is also called autotaxin (ATX). LPA interacts with specific G-protein coupled receptors and is involved in the regulation of cellular survival, proliferation, differentiation and motility. LPA also has roles in several pathological disorders, such as cancer and pulmonary, dermal and renal fibrosis. The involvement of the ATX-LPA pathway has recently been demonstrated in inflammatory responses and apoptosis of fibroblast-like synoviocytes (FLS) from patients with rheumatoid arthritis and during the development of experimental arthritis. This review summarizes the current literature of the ATX-LPA pathway in rheumatoid arthritis.

Keywords

Autotaxin, experimental arthritis, fibroblast-like synoviocytes, inflammatory response, lysophosphatidic acid, rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory, and systemic autoimmune disease that affects peripheral joints with a symmetric distribution. RA is characterized by chronic synovial inflammation that leads to the progressive destruction of cartilage and bone (Feldmann et al., 1996; Firestein, 2003; Klareskog et al., 2009). Within the peripheral joints, a severe inflammatory process leads to synovial hyperplasia that is caused by the proliferation of resident fibroblast-like synoviocytes and the recruitment of additional inflammatory cell-types. The infiltrating cells then produce a wide range of cytokines and chemokines that maintain a state of chronic inflammation within the joint that can lead to the destruction of the joint architecture without treatment (Feldmann et al., 1996; Firestein, 2003; Klareskog et al., 2009).

Recent studies have demonstrated the involvement of the ATX-LPA (Autotaxin-lysophosphatidic acid) pathway in the pathogenesis of RA. ATX is a secreted lysophospholipase D that produces LPA by cleaving the choline group from lysophosphatidylcholine (LPC). The increased expression of both ATX and LPA has been reported in synovial fluid and fibroblasts like synoviocytes (FLS), from RA patients (Santos et al., 1996; Kehlen et al., 2001; Nikitopoulo et al., 2012; Miyabe et al., 2013; Nochi et al., 2008). Several research groups have analysed the function of LPA in RA FLS. Nochi *et al.* described that LPA induced cytokines and COX-2 production in RA FLS. Additionally, Orosa *et al.* demonstrated that LPA receptor inhibition induced apoptosis in these cells. It has also been described that the genetic deletion of the LPA_1 receptor (Miyabe et al., 2013), ATX (Nikitopoulou et al., 2012) genes or LPA receptor inhibition (Orosa et al., 2012) reduced the severity of experimental arthritis. In this review we summarize the current literature of the ATX-LPA pathway in RA.

2. Synthesis and function of lysophosphatidic acid

ATX was initially identified in 1992 in human melanoma cells as a potent inducer of motility (Stracke et al., 1992). Ten years later, Tokumora et al. and Umezu-Goto et al. purified lysophospholipase D from plasma and foetal calf serum. They showed that this enzyme corresponded to ATX. ATX cleaves the choline group from lysophosphatidylcholine to produce the majority of extracellular LPA. This finding was demonstrated using heterozygous mice for *Enpp2*, which is the gene encoding ATX. The plasma ATX levels in these mice were reduced by 50% (van Meeteren et al., 2006; Tanaka et al., 2006). Furthermore, the transgenic ATX mice had a 2-fold increase in their plasma ATX levels (Pamuklar et al.,

2009). In contrast, it has been reported that intracellular LPA is produced by the deacylation of phosphatidic acid by phospholipase A1 and A2 (PLA1, PLA2) (Aoki et al., 2008).

LPA binds to six specific cell surface GPCR receptors. The receptors LPA_1 (lysophosphatidic receptor 1, formerly, Edg2), LPA_2 (Edg4) and LPA_3 (Edg7) belong to the endothelial differentiation gene family (EDG) and share sequence homology (50-60% amino acid homology) [17-19]. LPA_4 (formerly, GPR23/$P2Y_9$) (Noguchi et al., 2003), LPA_5 (GPR92) (Lee et al., 2006) and LPA_6 ($P2Y_5$) (Pasternack et al., 2008) are more dissimilar and closely related to P2Y nucleotide receptors. Furthermore, PPARγ has been identified as an intracellular receptor for LPA (McIntyre et al., 2003).

After binding to its receptor, LPA activates several signalling pathways through coupling to the G-proteins $G_{i/o}$, $G_{q/11}$ and $G_{12/13}$ depending on the cell type. The interaction with $G_{i/o}$ protein leads to the inhibition of adenylate cyclase and activation of phosphatidylinositol 3-kinase/Akt and Ras/MAPK pathways. The interaction with $G_{q/11}$ activates phospholipase C and protein kinase C, whereas LPA coupled to $G_{12/13}$ leads to the activation of RhoA GTPase (Noguchi et al., 2003; Lee et al., 2006; van Meeteren et al., 2007; Umezu-Goto et al., 2004. By controlling these pathways, LPA is able to regulate several cellular processes, including survival, proliferation, differentiation and motility (van Meeteren et al., 2007). In addition, the ATX-LPA pathway is involved in the growth and metastasis of different tumours (Umezu-Goto et al., 2004; Gotoh et al., 2012) and in other pathological disorders, such as pulmonary, dermal and renal fibrosis (Pradère et al., 2007; Johnson et al., 2002).

3. ATX-LPA pathway in rheumatoid arthritis

In RA, the synovial membrane becomes hyperplastic due to the proliferation of resident macrophages and FLS and the subsequent infiltration of circulating cells, such as T cells, B cells, monocytes, natural-killer cells, neutrophils, plasma cells and mast cells (Feldmann et al., 1996; Firestein, 2003; Klareskog et al., 2009). Resident FLS play an active role in synovial inflammation and damage. They secrete a plethora of cytokines and chemokines that perpetuate a state of chronic inflammation and produce matrix-remodelling enzymes, such as matrix metalloproteinases (MMPs), agrecanases and cathepsins that contribute to joint destruction. The production of these inflammatory factors is mediated by signal transduction pathways, such as the mitogen-activated protein kinase (MAPK) signalling pathway, which includes c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK) and

p38 MAPK (Inoue et al., 2006; Schett et al., 2008). All three phosphorylated forms of MAPK have been detected in RA synovium and cultured FLS (Schett et al., 2008; Thalhamer et al., 2008).

The involvement of the ATX-LPA pathway in the pathogenesis of rheumatoid arthritis was initially suggested by Santos et al. in 1996. In this work, the authors described the presence of ATX mRNA in FLS from RA patients and its downregulation by IFN-γ. The same group later performed a comparative analysis and found that levels of ATX mRNA in FLS from RA patients were 5-fold higher than non-RA patients (Kehlen et al., 2001). A recent study from Aidinis' group confirmed the presence of ATX in FLS from RA patients and reported that this expression was higher in FLS adjacent to damaged cartilage (Nikitopoulou et al., 2012). Furthermore, Miyabe et al. recently showed a higher ATX expression in RA synovium compared with OA synovium. In addition, two research groups demonstrated the presence of ATX protein in synovial fluid from RA patients (Nochi et al., 2008; Zhao et al., 2007). In the study of Nochi et al., the authors found that the ATX substrate LPC was also present in synovial fluid from RA patients. Collectively, these findings suggest that ATX could be involved in the pathogenesis of rheumatoid arthritis by inducing local production of LPA in the synovium and synovial fluid from LPC. However, infiltrating cells in synovium such as T cells, neutrophils and monocytes could contribute to the increase of LPA in synovial fluid and synovium of RA patients because LPA can be also produced intracellularly by hydrolysis of phosphatidic acid (PA) by cytosolic phospholipases.

. Phospholipases cleave membrane phospholipids from inflammatory cells, activated platelets, erythrocytes, adipocytes and cancer cells (Aoki et al., 2008; Neidlinger et al., 2006).

Several reports have analysed the role of LPA and its receptors in the pathogenesis of RA. Zhao et al. reported that LPA_1, LPA_2 and LPA_3 mRNAs were detected in RA FLS, whereas LPA_4 and LPA_5 were not expressed. LPA_1 was the main expressed receptor, followed by LPA_3, and LPA_2 showed the lowest expression. Nochi et al. confirmed these findings, and two recent reports demonstrated that LPA_1 expression in RA FLS was significantly higher than the expression in FLS from patients with osteoarthritis (Miyabe et al., 2013; Orosa et al., 2012). More recently, Miyabe et al. have demonstrated the expression of $LPA_{4\text{-}6}$ receptors in RA FLS (Miyabe et al., 2014).

In addition to these expression analyses, the functional role of LPA signalling in inflammatory responses of RA FLS has been extensively studied. It has been demonstrated that LPA binds to receptors on RA FLS and through $G_{i/o}$ and $G_{12/13}$ proteins, induces the activation of MAPK/ERK and Rho signalling

pathways. These pathways, in turn, lead to activation of NFkB transcription factor, which regulates the production of cytokines and chemokines (Figure 1). By using antagonists of $LPA_{1/3}$ receptors Zhao et al. demonstrated the role of LPA in the secretion of IL-6 and IL-8 and the synergy between LPA and TNF-α in the production of theses cytokines (Zhao et al., 2008). The main receptor involved was LPA_3 and the signalling pathways were p38, pERK MAPK and Rho kinase (Zhao et al., 2008). The role of LPA in the production of COX-2 in RA FLS and the synergistic effect of LPA and IL-1β on COX-2 induction was described by Nochi et al. This effect was inhibited by treatment with Ki16425, which is a specific $LPA_{1/3}$ receptor antagonist. (Nochi et al., 2008). More recently, Miyabe et al. have reported the suppression of LPA-induced proliferation of RA FLS using a new selective LPA1 receptor antagonist, LA-01. This antagonist also reduced the LPA-induced production of IL-6, CCL-2, VEGF, MMP-3 and the expression of adhesion molecules, VCAM and ICAM in these cells (Miyabe et al., 2014).

Other effects of LPA that have also been demonstrated include stimulation of the migration of RA FLS (Zhao et al., 2007; Miyabe et al., 2014) and of human mesenchymal stem cells (hBMSCs) (Song et al., 2010). In a first study, Zhao et al. reported that LPA induced the migration of FLS from RA patients in an *in vitro* wound-closing assay. This effect was mediated by the LPA_1 receptor and was regulated by p38 MAPK and Rho kinase (Zhao et al., 2007). In the same way, Miyabe et al. reported that LA-01 antagonist suppressed the LPA-induced migration of RA FLS. Completing this panorama, Song et al. showed that synovial fluid from RA patients was able to induce the migration of hBMSCs through a Boyden chamber and LPA from synovial fluid activated the LPA_1 receptor to cause this effect (Song et al., 2010). Interestingly, Miyabe et al. reported that LPA promote pseudoemperipolesis (which is the *in vitro* migration of cells beneath other cells of different lineage) of $CD4^+$, $CD8^+$ T and $CD19^+$ B cells beneath RA FLS and this effect was suppressed after treatment with LA-01 (Miyabe et al., 2014)..

4. LPA signalling pathway in synovial apoptosis

In rheumatoid arthritis, the imbalance between cell proliferation and apoptosis is pivotal for the accumulation of FLS, which contributes to synovial hyperplasia. RA FLS are resistant to apoptosis despite the expression of functional death receptors, such as Fas/CD95, TRAIL-R1, TRAIL-R2 and TNFR. In RA, FLS are strongly influenced by a plethora of cytokines, including TNF. TNF is able to induce proliferation and apoptosis in addition to its effect as an inflammatory mediator. However, in RA FLS, TNF induces proliferation and inhibits apoptosis (Aggarwal, 2003; Smeets et al., 2003; Orosa et al., 2014).

The effect of LPA_1 loss on TNF-induced proliferation and apoptosis of RA FLS has been assessed to determine whether LPA has a role during synovial hyperplasia (Orosa et al., 2012). The authors found that suppression of the LPA_1 receptor in RA FLS abrogated the proliferation induced by TNF and sensitized the cells to TNF-induced apoptosis. This effect was associated with the upregulation of the apoptotic genes TRADD, TRAIL and PYCARD (Orosa et al., 2012). Interestingly, *lpa₁* receptor suppression did not reduce the inflammatory response of RA FLS to TNF, but rather increased the production of IL-6, IL-8 and MCP-1. This higher inflammatory response was accompanied by an increase of p38, pERK and pJNK activation (Orosa et al., 2012). Collectively, these results suggest interplay between TNF and LPA_1 signalling pathways (Figure 1) and indicate that LPA_1 is a therapeutic target to block synovial hyperplasia.

5. ATX-LPA pathway in animal models of rheumatoid arthritis

Several animal models have been used to characterize the mechanisms involved in the pathogenesis of arthritis and to test new therapeutic strategies. The collagen-induced arthritis (CIA) model (Courtenay et al., 1980; Luross et al., 2001; Hegen et al., 2008), the K/BxN model (Kouskoff et al., 1996; Kouskoff et al., 1997) and TNF transgenic mice (Keffer et al., 1991) are the most used animal models. In the CIA model, DBA/1 mice with the MHC Class II I-A^q haplotype develop arthritis after injection with type-II collagen in complete Freund's adjuvant. The initial injection is followed by an intraperitoneal collagen booster 21 days later. This model allows the study of the two phases in the development of arthritis. The initial autoimmune response involves the production of collagen-specific T and B cells. The subsequent effector phase is characterized by joint inflammation, cartilage damage and bone erosion. The disease severity peaks at day 35 and the incidence and severity of the disease depends on mice maintenance conditions and environment (Courtenay et al., 1980; Hegen et al., 2008). In the K/BxN arthritis model described by Kouskoff et al., a T cell receptor (TCR) transgene recognizes glucose-6-phosphate isomerase presented in the context of the MHC class-II molecule I-A^{g7}. The immune reaction induces an early and rapidly progressive arthritis that is T- and B-cell dependent and is similar to human RA. The serum from these mice causes arthritis in a wide range of recipient strains because of high levels of autoantibodies against the glucose-6-phosphate isomerase (GPI) and represents the K/BxN serum-transfer model. This model allows the study of the mechanisms and molecules involved in the effector phase of arthritis independent of the initial autoimmune response (Kouskoff et al., 1997). The

TNF transgenic mouse reported by Keffer et al. in 1991 was the first model of spontaneous arthritis. These mice overexpress human TNF and spontaneously develop an erosive chronic poly-arthritis that closely mimics human RA. The mice develop synovial hyperplasia, pannus formation, cartilage destruction and bone erosion. This model established the fundamental role of TNF in the pathogenesis of RA. Recently, these three models have been used by several research groups to analyse the involvement of the ATX-LPA pathway in experimental arthritis models (Table 1).

Table **1**. Studies showing reduced clinical arthritis, synovial inflammation and hyperplasia in experimental models by interfering with the ATX-LPA.

Arthritis Model	Treatment	Genetic Approach	Additional Findings*	References
CIA	BrP-LPA (LPA receptor antagonist and ATX inhibitor)	-	Disease incidence reduction	(Nikitopoulou et al., 2013)
K/BxN serum transfer model	Ki16425 (LPA_{1-3} antagonist)	-	Increased synovial apoptosis and osteoblast differentiation; reduction of osteoclast differentiation.	(Orosa et al., 2014)
CIA	LA-01 (LPA_1 antagonist)	-	Reduction of osteoclast formation and suppression of Th17 cells.	(Miyabe et al., 2013)
CIA	-	LPA_1 Knockout mice	Total protection against arthritis. Suppression of differentiation of Th17 cells and osteoclast formation.	(Miyabe et al., 2013)
hTNF/CIA	-	Conditional genetic ATX ablation in mesenchymal cells	-	(Nikitopoulou et al., 2012)

In the first of these studies, Nikitopoulou et al. analysed the TNF transgenic and the CIA models in FLS lacking ATX by conditional ablation. The authors showed that in the two models the absence of ATX reduced the clinical severity of arthritis and the synovial inflammation and hyperplasia using a histopathological analysis of joints. More recently, Orosa et al. analysed the effect of lysophosphatidic acid receptor inhibition in the K/BxN serum-transfer arthritis model. In this work, mice were injected

with K/BxN serum and treated with the $LPA_{1/3}$ antagonist, Ki16425. This treatment attenuated the clinical severity of arthritis and reduced synovial inflammation, cartilage damage and bone erosion in joints from arthritic mice. These findings were accompanied by increased apoptosis and the reduced production of inflammatory mediators in joints. Interestingly, the marked decrease of bone erosion was the result of reduced osteoclast differentiation and function with increased differentiation of osteoblasts and bone mineralization (Orosa et al., 2014). Furthermore, Miyabe et al. reported that LPA_1 deficient mice were protected from arthritis after immunization with type II collagen (Miyabe et al., 2013). The histopathological analysis of joints showed an absence of synovial inflammation, cartilage damage and bone erosion. Interestingly, the authors found interplay between the LPA_1 receptor and Th17 cells as differentiation of these cells was abrogated in mice lacking LPA_1 (Miyabe et al., 2013). This study also reported the reduction of osteoclast differentiation in LPA_1 deficient mice, which is consistent with findings from Orosa et al. Thus, it seems clear that ATX-LPA signalling pathway plays a direct role in osteoclast differentiation. Additional evidence supporting this role comes from studies in other fields as the study of David et al. reporting that expression of ATX controls the progression of osteolytic bone metastasis in mice and that LPA increases osteoclast formation in response to macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kB ligand (RANK-L) (David et al, 2010). Furthermore, Lapierre et al. have recently reported that LPA increases osteoclast survival (Lapierre et al., 2010).

In addition to the data provided by the Ki16425 treatment in the K/BxN arthritis model, new findings from the study of two new LPA receptor antagonists confirm the involvement of the LPA pathway in the pathogenesis of experimental arthritis. In the study by Miyabe et al., the authors reported the treatment of mice with CIA using the specific LPA_1 antagonist LA-01, which reduced the incidence and severity of arthritis, synovial inflammation and bone erosion. Additionally, the LPA analogue BrP-LPA acts as a pan-antagonist of LPA receptors and as an inhibitor of the lysophospholipase D activity of ATX. The treatment with BrP-LPA also reduced the incidence and severity of arthritis in mice injected with type II collagen (Nikitopoulou et al., 2013).

6. ATX-LPA pathway inhibitors

ATX and LPA_1 receptor have been involved in several pathological conditions besides rheumatoid arthritis, such as cancer, neuropathic pain, fibrotic diseases, etc. Development of ATX and LPA receptor inhibitors have been initiated some years ago with treatment of these diseases in mind. The early synthetic ATX inhibitors were lipid analogues of LPA or other lipids derived from the fact that LPA and S1P are feedback inhibitors of ATX. Later, screening of libraries led to discovery of new small-molecule leads such as the potent ATX inhibitor PF8380 which lead to a reduction of > 95% of LPA in rat plasma. In addition, the boronic acid derivatives were also potent ATX inhibitors. However, in our knowledge, no ATX inhibitor has reached clinical trials due to problems with*in vivo* stability and bioavailability. Regarding LPA receptor inhibitors, several $LPA_{1/3}$ antagonists have been developed, among them,Ki16425, a potent $LPA_{1/3}$ antagonist that reduced the induced renal fibrosis in mice (Pradère et al., 2007), the progression of bone metastasis in an experimental model (Boucharaba et al., 2006) and the severity of arthritis and bone erosion in mice (Orosa et al., 2012). Later, Debiopharm developed a R-stereoisomer of Ki16425, Debio 0719, which inhibited the formation of lung and bone metastases in a preclinical breast cancer mouse model (David et al., 2012). However, in our knowledge, neither these two compounds has entered yet clinical trials. More recently, Amira Pharmaceuticals has developed potent LPA_1 selective antagonists that inhibit lung fibrosis in the mouse bleomycin model. In fact, one of these drugs acquired by Bristol-Myers Squibb is the first inhibitor (BMS-986020) of this pathway that is already in clinical trials (NCT 01766817) for the treatment of idiopathic pulmonary fibrosis. We hope that new ATX or LPA_1 receptor antagonists with high potency and good pharmacological profile could reach clinical trials in RA and determine whether ATX-LPA pathway is a useful target in this disease.

7. Concluding remarks

This review has summarized the intense research demonstrating the role of the ATX-LPA pathway in the pathogenesis of rheumatoid arthritis. Overall, data reviewed here point to ATX-LPA_1 receptor pathway as a therapeutic target in this disease. Blocking ATX-LPA pathway could obtain clear therapeutic benefits involving multiple mechanisms, including reduction of FLS proliferation, cellular infiltration and sensitization of FLS to apoptosis, leading in turn, to reduction of synovial hyperplasia, key in the pathogenesis of rheumatoid arthritis. Other mechanisms include reduction of inflammatory mediators

produced in the synovia and, very interestingly, a marked decrease of bone erosions that otherwise could lead to irreversible deformities and disability.

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Conflict of interest

There is no conflict of interest in the publication of this review article.

Figure Legends

Figure 1. Schematic representation of the effects of LPA signalling in fibroblast-like synoviocytes from rheumatoid arthritis. The binding of LPA to LPA_1 and LPA_3 receptors in RA FLS activates p38, ERK1/2 and Rho pathways via Gi/o and G12/13 leading to production of chemokines, cytokines, metalloproteinases and adhesion molecules, and inducing cell migration. In addition, the binding to LPA_1 receptor also contributes to apoptotic response mediated by death receptors in RA FLS by regulating expression of TRAIL, TRADD and PYCARD.

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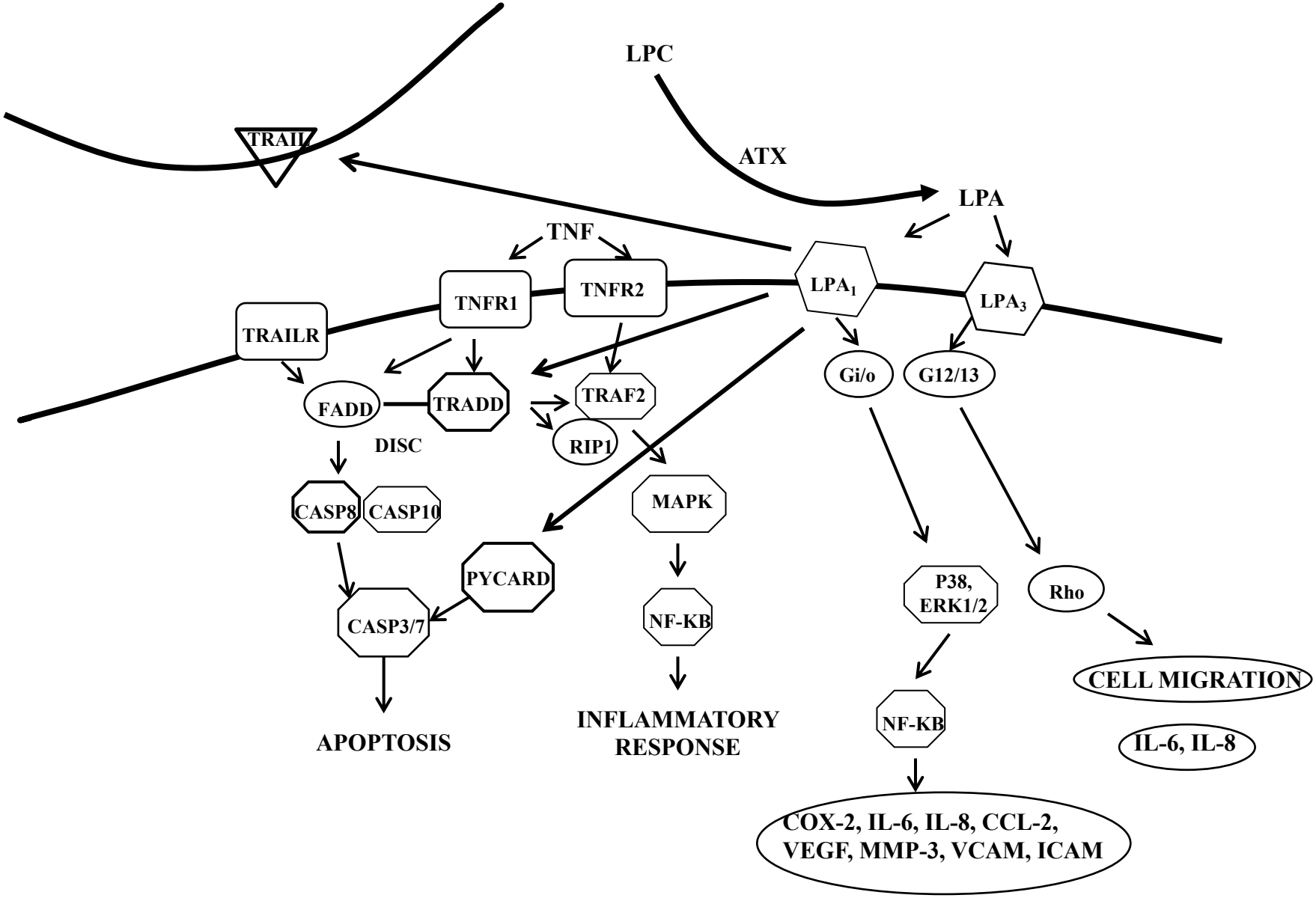
LPC
TRAIL
ATX
LPA
TNF
TNFR1
TNFR2
LPA1
LPA3
TRAILR
FADD
TRADD
TRAF2
RIP1
DISC
Gi/o
G12/13
CASP8
CASP10
MAPK
PYCARD
CASP3/7
NF-KB
P38,
ERK1/2
Rho
CELL MIGRATION
IL-6, IL-8
NF-KB
APOPTOSIS
INFLAMMATORY
RESPONSE
COX-2, IL-6, IL-8, CCL-2,
VEGF, MMP-3, VCAM, ICAM

Figure 1