The biological role of Tribbles proteins in the inflammatory responses of monocytes. The link between inflammation and atherosclerotic signals

Katalin Éder

Ph.D. Thesis

Department of Medical Microbiology and Immunobiology
Faculty of Medicine, University of Szeged
Szeged, Hungary

2008

Introduction

In Western societies, some of the most common causes of death are the various complications of atherosclerosis. Many known genetic and environmental risk factors contribute to the development of atherosclerosis. These include elevated levels of modified low density lipoproteins (LDL), free radicals arising from oxidative stress and cigarette smoking, obesity, hypertension, diabetes mellitus, chronic infections and obviously combinations of these or as yet unrecognized factors.

Atherosclerosis is a multifactorial, complex pathological process. Cell-cell and cell-matrix interactions and communications involving macrophages, vascular smooth muscle cells, vascular endothelial cells and lymphocytes are all likely to be involved. Monocytes, platelets, T lymphocytes and two cell types of the artery wall, endothelial and smooth muscle cells (SMC) interact in multiple ways to generate atherosclerotic lesions .

Atherogenesis appears to depend on the local presence of high amounts of modified LDL. Normally, LDL is transported through the LDL receptors, which is a feedback inhibited process by the accumulation of cholesterol within the cell. However, chemically (most frequently oxidized) or enzymatically modified LDL can not be transported by LDL receptor.

Modified LDL has the capacity to induce the expression of inflammatory factors, in response to which monocytes migrate into the intima and differentiate into resident macrophages. Macrophages are able to take up the modified LDL by scavenger receptors (SR).

This unregulated uptake of modified LDL forms leads to foam cell formation. At the early stage of atherosclerosis foam cells form clusters. The intensive aggregation of these cells leads to the formation of the atheromatous core, and to the development of fatty streak lesions. This process is accompanied by the migration of smooth muscle cells into the intima. They proliferate, and secrete extracellular matrix proteins that form a fibrous plaque.

This phase of plaque development is influenced by interactions of macrophages and T cells and results in both humoral and cellular responses. Similarly, macrophages, endothelial cells and smooth muscle cells are activated and produce pro-inflammatory cytokines, such as TNF-alpha, IL-6, MCP-1 or IL-8. If prolonged, these inflammatory processes can induce the cell death of lipid-loaded macrophages and smooth muscle cell by apoptosis or necrosis.

The above processes lead to the formation of the necrotic core and the accumulation of extracellular cholesterol. Around the necrotic core, macrophages and macrophage foam cells produce and release metalloproteinases that facilitate plaque destruction. Plaque rupture exposes blood components to tissue factor, initiating coagulation, the recruitment of platelets, and the formation of a thrombus.

All these steps of atherosclerosis are controlled by several cytokines and chemokines. Numerous cytokines have been shown to induce monocyte recruitment, cell migration or alter the scavenger receptor and adhesion molecule expression. They also regulate the immune responses; they have an influence on dendritic cell maturation and Th1/Th2 development. Cytokines generate cellular responses through activation of intracellular signal transduction networks. In atherosclerosis, the NF-κB and Mitogen Activated Protein Kinase (MAPK) pathways are amongst the most prominent ones.

MAPKs are evolutionary conserved enzymes connecting cell-surface receptors to critical regulatory targets within cells. MAPKs regulate cell proliferation, control cell survival,

death and cell motility. It has been demonstrated that activation of MAPK cascades occurs in response to a wide range of stimuli, including pro-inflammatory cytokines, growth factors, mechanical stimuli (stress) and integrin dependent cell/matrix interactions. Increased MAPK activity has been reported in a number of pathogenic conditions, including cardiovascular cellular responses. The signalling network comprises of a three level kinase cascade: MAPKKK (MEKKs and MLKs, for instance), MAPKK (MKK3, 6, 4, 7 and MEK1) and MAPK (p38, JNK and ERK). Three parallel cascades are now commonly described: p38, the extracellular signal regulated protein kinases (ERK), and stress activated protein kinase/c-Jun N-terminal kinases (JNK). A large body of literature suggests that MAPKs may be organised into multiprotein complexes to create a functional signalling module. This organisation modulated by scaffold proteins, which interact with each of the protein kinases and control the spatial organisation of MAPK signalosomes.

Our group described the important role of a recently identified family of proteins, tribbles, in the signal processes of monocytes and smooth muscle cells. Tribbles are a novel family of proteins with MAPK modulator, scaffold-like function. *Tribbles* was first identified in Drosophila as a gene that is required for gastrulation, oogenesis, and viability. In humans there are three tribbles homologues, TRB-1,-2,-3. TRB-3 is the best studied member of the mammalian tribbles family. Identification of this protein was first reported in 1999. Mayumi- Matsuda and colleagues described it as a gene that is up-regulated in a neuronal cell line undergoing nerve growth factor withdrawal induced apoptosis. TRB-2 was originally identified as a transiently expressed, mitogen induced, and highly labile cytoplasmic phosphoprotein, induced by thyroid hormone in dog thyroid cells. Human TRB-1 was first identified as a homologue of TRB-2.

Based on amino acid sequence, tribbles resembles a serine-threonine kinase; however, it has a variant catalytic core that lacks a canonical ATP binding site, and their function is unknown. Tribbles proteins consist of three domains: N-terminal domain, kinase-like domain and C-terminal domain. Tribbles kinase-like domain is located in the middle of the protein, with relatively short N- and C-terminal flanking sequences.

Many aspects of tribbles action appear to be cell type and/or organism specific. It has been shown that tribbles are able to bind to various MAPKKs and that their concentration regulates preferential activation of the different MAPK pathways, presumably leading to different cellular responses.

The aim of my studies was to elucidate the biological effects of a novel family of MAPK regulators, tribbles, in inflammatory monocyte function in the context of atherosclerosis.

Hypothesis and aims of the study

The hypothesis to be tested by my studies was that tribbles proteins act as regulatory molecules in inflammatory processes through modulation of MAPK signalling. Thus they may have a role in atherogenesis, especially in the inflammation induction role of macrophages.

Aims of my studies were to investigate the role of human tribbles, particularly human TRB-2, in human monocyte cells function *in vitro* and to elucidate related molecular mechanism by which TRB interact with protein kinases and regulate activity of binding partners or activation of down stream protein molecules.

We focused on the following questions:

- the uptake of modified LDL molecules by monocyte
- the role of MAPK pathways and tribbles in LDL uptake by monocytes
- the effect of modified LDL molecules on Trb-2 expression level
- the effect of TRB-2 on LPS induced IL-8 production
- and the role of the MAPK pathways in IL-8 production.

Materials and methods

Ethics:
Human samples were obtained under the ethical approval granted by the North Sheffield Research Ethics Committee. This study conforms to the principles outlined in the Declaration of Helsinki.
Cell culture:
Human monocytic leukaemia THP-1 cells were maintained in RPMI supplemented with 10% foetal calf serum (FCS), L-glutamine and penicillin-streptomycin.
THP-1 transfection with DharmaFECT
Transfections were performed using DharmaFECT 2 transfection according to the manufacturer's recommendation. For experiments $1{,}7x10^5$ cells were used on 6 well plates, per transfection.
THP-1 electroporation with siRNA
Transfections were performed using Amaxa Nucleofector. For most experiments, 1.0×10^6 cells were used per nucleofection. siRNA SmartPool against human *TRB-1,2,3* and *MAPKK*s were purchased from Dharmacon and used according to the manufacturer's recommendation.
ELISA:
THP-1 cells were transfected by specific siRNA, stimulated for 36 h by 100 ng/ml LPS and the production of IL-8 was measured by ELISA.
LDL uptake
Monocyte uptake of acetylated LDL was evaluated by flow cytometry using lipoproteins labelled with the fluorescent probe Dil (1,1'-Dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate). Fluorescence of cells were examined on FACScan cytometer. The Dil fluorescence was recorded on channel FL2 and analyzed by the CellQuest program.
MAPK inhibitor treatment
MEK1 inhibitor, p38 MAPK inhibitor and JNK MAPK inhibitor were purchased from Calbiochem and used at 20 μM for MEK1 and JNK MAPK inhibitors and 0.2 μM for p38 MAPK inhibitor. The cells were treated for 1 h with inhibitors before the LPS treatment.
RNA isolation and Quantitative Real-time PCR analysis
Total RNA was extracted from whole blood of patients with unstable angina and chronic stable angina using QIAamp RNA blood minikit according to the manufacturer's description. *TRB-2*, and *β–actin* genes were analyzed by Quantitative real time PCR. To quantify transcript levels of the *TRB-2* gene, *β–actin* was used as a house keeping control, and each sample was normalized with respect to its *β–actin* transcript content.
Statistical Analysis
Experimental data was analysed by the PRISM (GraphPad) package, using the appropriate tests, as indicated in the figure legends.

Results

LDL uptake

Previous data show that activation and subsequent recruitment of monocytes to the developing lesion of the arterial wall has an important role in the progression of atherosclerosis. Monocytes internalise LDL particles and this process leads to differentiation of monocytes into foam cells, which produce cytokines and chemokines. MAPK signalling pathways, which are involved in inflammatory responses and LDL uptake by monocytes, can be modulated by tribbles.

We first examined the putative cell activators that would affect LDL uptake. We treated the cells with 100 ng/ml LPS or 50 ng/ml PMA for 24 h. After incubation we added 5 μg/ml Dil-acLDL to the cells. Following 4 h incubation we measured the fluorescence of THP-1 cells by flow cytometry. LDL uptake was enhanced only by LPS.

To determine whether the fluorescence of cells was due to an active uptake or passive cell surface binding of LDL, we incubated cells with Dil-acLDL at 37^{o}C or 4^{o}C for 4h. As the uptake is an active process which only occurs at 37^{o}C, whilst binding can be readily detected at 4^{o}C. As negative control, we added unlabelled LDL to cells also. Uptake was quantified by flow cytometry. Fluorescence was enhanced in the 37^{o}C treated cells only.

To characterize the dynamics of acLDL uptake by THP-1, LDL was added to the cells for 1, 2, 4 and 16 h. The activation of cells was achieved using 100 ng/ml LPS. There was a significant difference between LPS treated and non treated samples at each time point with the highest uptake observed after 16 h. The latter time point was therefore used in subsequent experiments. To determine optimal dose of LDL we added 5, 10 and 15 μg/ml Dil-acLDL to cells for 4 h. The difference between the uptake of LPS treated and non-treated cells was significant at all doses. We therefore used the lowest concentration for subsequent experiments.

Role of MAPK pathway in acLDL uptake by THP-1 cells

Several reports suggest that expression of scavenger receptors is regulated via the MAPK pathways. However, the link between LDL uptake, inflammatory activation and MAPK signalling in monocytes is much less established. Therefore, the effect of pharmacological inhibition of specific MAPKs on acLDL uptake was tested. Cells were treated with MAPK inhibitors in the presence or absence of LPS (Figure 1). The results show that blocking the activation of JNK pathway lead to a marked elevation of acLDL uptake that was independent of inflammatory stimulation. Whilst the ERK and p38 pathways do not influence the uptake of acLDL under non-inflammatory conditions, both pathways led to a significant decrease in acLDL uptake after LPS stimulation. These observations demonstrate that the enhanced capacity to take up acLDL by inflammatory activated THP-1 cells is mediated by specific MAPK pathways.

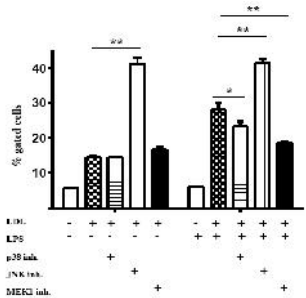

Figure 1. **acLDL uptake is regulated by MAPK signalling pathways.** Blocking the activation of JNK pathway lead to a marked elevation of acLDL uptake, this is independent of inflammatory stimulation. Whilst the ERK and p38 pathways influence the LDL uptake only under inflammatory conditions.

The effect of knockdown of tribbles on the uptake of acLDL in THP-1 cells

As stated above, tribbles regulate MAPK activation. The effect of tribbles on LDL uptake was therefore tested. THP-1 cells were transfected with siRNA against *TRB-1,-2,-3* using lipotransfection methods (DharmaFECT). After transfection Dil-labelled acLDL was added to cells and LDL uptake measured by FACS. There were no significant differences in LDL uptake between si*TRB*s or siNc transfected cell.

To determine whether another siRNA transfection method would produce similar results, cells were electroporated with Nucleofector kit. This resulted similar effects and it can be concluded that reduced tribbles level do not affect the LDL uptake. (Fig. 2. a, b)

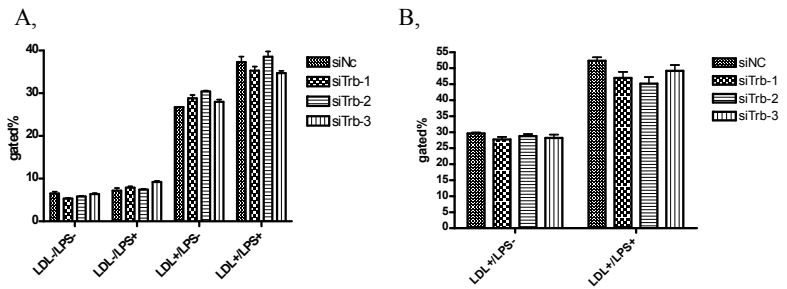

Figure 2. **si*TRB-2* lipofection (A) and electroporation (B) do not modulate the LDL uptake of THP-1 cells**. THP-1 cells were transiently transfected with control (siNC) or tribbles specific siRNA. The impact of reduced tribbles levels on acLDL uptake was measured in the presence or absence of LPS, as previously.

The effect of overexpression of tribbles on acLDL uptake

To complement the knockdown studies, THP-1 cells were transiently transfected with control or tribbles expression constructs and incubated with acLDL, in the presence or absence of LPS, as above. Similar to results obtained in knockdown experiments, overexpression of these proteins did not affect the ability of cells to take up acLDL.

The effect of LDL on LPS induced IL-8 production and tribbles expression

Tribbles expression does not appear to regulate LDL uptake in THP-1 cells. In order to characterize the contrary effect we investigated the role of LDL uptake on cytokine and tribbles expression.

To investigate the effect of LDL uptake on cytokine expression, THP-1 cells were treated with or without acetylated LDL (acLDL) after LPS stimulation, and the amount of IL-8, as a biologically relevant marker of inflammatory activation of monocytes, was measured by ELISA. The uptake of acLDL significantly enhanced IL-8 production. (Fig.3.)

TRB-2 mRNA expression levels were also measured by using qRT-PCR and were found to be down-regulated by acLDL after 4 h and more efficiently after 24 h. (Fig.4.a,)

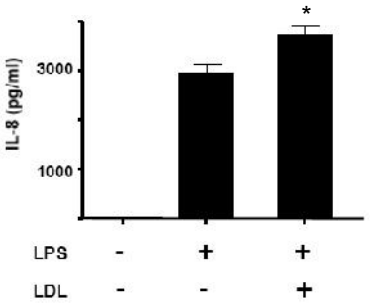

Figure 3. **AcLDL potentiates LPS induced IL-8 production.** The impact of 24hrs acLDL and LPS treatment alone and in combination was studied on IL-8 protein production in THP-1 cells. Cells were lysed and IL-8 levels were quantified by ELISA. One way ANOVA with Dunnett's Multiple Comparison Test was performed to analyse the results. *: $p<0.05$

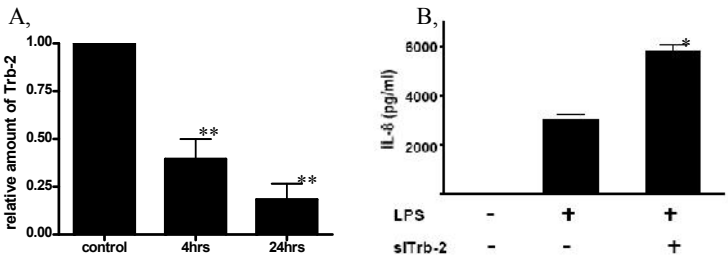

Figure 4. (A) **In THP-1 cells *TRB-2* mRNA level was down-regulated by acLDL treatment** after 4h and more efficiently after 24hours. *TRB-2* expression was measured in response to acLDL treatment by qRT-PCR. In order to assess statistical significance, one way ANOVA test with Dunnett's Multiple Comparison Test was performed. **: $p<0.01$ (B) **Reduced TRB-2 level enhanced the IL-8 production.** The impact of reduced TRB-2 levels on LPS induced IL-8 production was measured by ELISA. Student's t-test was performed to analyse the results. $p=0.041$

Role of JNK and ERK pathways in modulation of IL-8 production by TRB-2

To examine the effect of TRB-2 on cytokine expression, TRB-2 levels were suppressed by transfection of siRNA against TRB-2 and IL-8 release was measured. IL-8 production was induced in siRNA transfected cells by LPS treatment, as above. The results in Figure 4.b, show that si*TRB-2* treated THP-1 cells produce significantly higher levels of IL-8, compared to cells transfected with control siRNA.

Mitogen-activated protein kinases (MAPK) playing a role in regulating cell growth, migration and differentiation, are implicated in the development of atherosclerosis. Thus MAPKs, p38 MAPK and ERK 1/ 2, and JNK 1/2 appear to be implicated in the regulation of IL-8 by human macrophages. In addition previous data suggest tribbles to be mediators of MAPK signalling.

Selective MAPK inhibitors were used to block individual pathways and investigated upon their involvement in IL-8 production by THP-1 cells. These data demonstrate that blocking of JNK and ERK but not p38 pathways suppresses IL-8 production by monocytes. (Fig.5.)

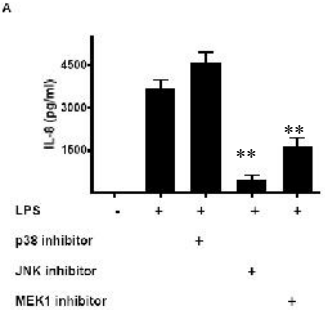

Figure 5. **The involvement of MAPK pathways in LPS induced IL-8 production** was measured by the use of inhibitors of specific MAPK pathways. Blocking of JNK and ERK but not p38 pathways suppresses the IL-8 level of monocytes. Cells were lysed and IL-8 levels were quantified by ELISA. One way ANOVA with Dunnett's Multiple Comparison Test was performed to analyse the results. **: $p<0.01$

TRB-2 modulation of IL-8 production via interaction with MAPKKs

Previously, we demonstrated that tribbles-1 and -3 proteins interact with MAPKKs and regulate their activity. Therefore, the interaction of TRB-2 with MKK4/SEK-1, MKK7 and MEK-1, which are known activators of JNK or ERK, was investigated. The results show that down-regulation of kinase levels led to impaired IL-8 production. These observations are compatible with a negative regulatory role for TRB-2 in control of ERK and JNK activation (Fig.6.)

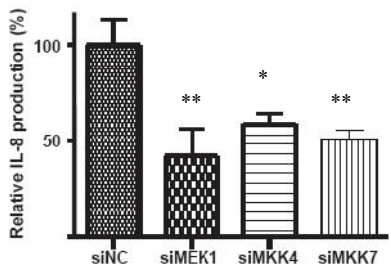

Figure 6. **Down-regulation of MAPKK levels attenuate IL-8 production**.THP-1 cells were transfected with si*MAPKK* or control siRNA (siNC), as indicated and stimulated by LPS. Production of IL-8 was detected by ELISA. One way ANOVA with Dunnett's Multiple Comparison Test was performed to analyse the results. *: $p<0.05$, **: $p<0.01$

Relationship of TRB-2 levels and IL-8 produced in response to LPS in primary monocytes

In order to investigate the potential *in vivo* relevance of our findings, responsiveness of primary monocytes from four healthy volunteers was assessed by stimulating cells with LPS or with the combination of LPS and acLDL. acLDL increased the amount of IL-8, produced in response to LPS in a manner similar to that seen in monocytic cell lines. (Fig.7.a) Furthermore, the level of IL-8 inversely correlated to the expression of TRB-2 in primary monocytes. (Fig.7.b)

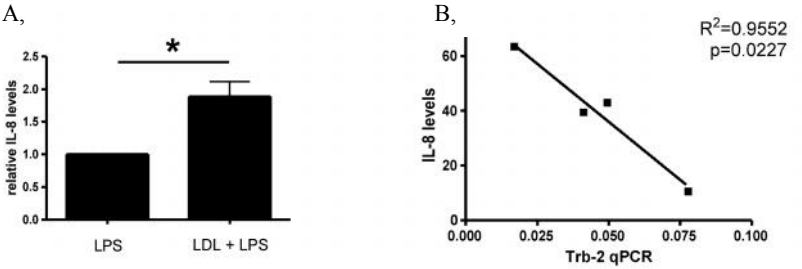

Figure 7. **TRB-2 levels are inversely correlated to the IL-8 produced in response to LPS in primary monocytes** (A) The ability of human primary monocytes to produce IL-8 was assessed. acLDL increased the amount of IL-8 produced in response to LPS (B) The relationship between the amount of IL-8 produced (LPS+acLDL treatment) and *TRB-2* expression was measured by linear regression.

Discussion

Elevated serum LDL levels have been implicated as a major risk factor for atherosclerotic vascular disease. Expression of inflammatory cytokines, and chemokines by a variety of vascular cells, furthermore proliferation of vascular smooth muscle cell, and migration of monocytes have all been linked to increased levels of modified LDL. The uptake of modified LDL by monocytes and differentiation to foam cells is a hallmark of the development of atherosclerotic lesions/plaques. A number of studies have demonstrated that MAPK pathways play a central role in both of these processes. Whilst the involvement of these MAPK pathways is relatively well characterised, the molecular mechanisms that modulate the ability of monocyte/macrophages to respond to inflammatory stimuli in an LDL-dependent fashion, remain imprecisely understood. Therefore, the involvement of inflammatory signals in regulating the uptake of modified LDL by monocytes and the role of TRB-2 in monocyte biology has been investigated. The functional links between LDL and inflammatory activation of monocytes has also been explored.

In line with previous findings, inflammatory activation of THP-1 cells via LPS appears to substantially enhance the uptake of acLDL. Pharmacological inhibition of MAPK signalling pathways demonstrates that these signalling systems play a differential role in modulating acLDL uptake. However our results demonstrate that acLDL uptake in THP-1 cells is tribbles-independent.

The data show clearly that TRB-2 is a novel regulator of MAPK signalling in the context of LPS-induced IL-8 release from a monocytic cell line and human peripheral blood monocytes. AcLDL uptake by monocytes is associated with a reduction in TRB-2 and

potentiates LPS-induced IL-8 release. This effect was inhibited by pharmacological inhibition of the ERK and JNK pathways. In line with our previous findings that tribbles interact with MAPKKs and regulate their activity, down-regulation of MEK1 and MKK4, -7 expression levels led to impaired IL-8 production in response to LPS.
These data indicate that *TRB-2* is an important negative regulator of monocyte IL-8 production in response to LPS and control the augmentation of this response by acLDL (Figure 8). This is in keeping with the findings of others which support the mechanism of action of tribbles through physical interaction with other signal transduction proteins. Recent reports demonstrate that TRB-3 is able to interact with and modulate the activity of a number of key inflammatory signalling mediators, including p65/RelA, ATF4 and Akt.

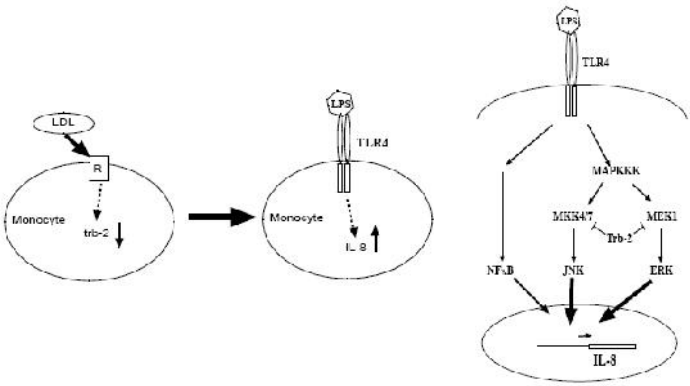

Figure 8. **A model for the role of TRB-2 in monocytes biology in inflammatory settings.** (A) acLDL uptake by monocytes triggers reduction of TRB-2 expression, resulting in a hypersensitive state towards inflammatory stimuli, as exemplified by LPS produced IL-8 production. **(B)** The molecular basis of TRB-2 regulatory function of MAPKK pathways in the expression of IL-8.

Increasing data indicate a convergence of innate immunity to pathogens and atherosclerosis. Several PAMPs can ligate TLRs and/or ScavRs. Some of these ligands participate not only in microbial pathogenesis, but in atherogenesis as well. It has been shown that nonspecific (endotoxin) stimulation of the immune system accelerates atherosclerosis in rabbits on a hypercholesterolemic diet. Some evidence has implicated microbial pathogens in human atherogenesis. Bacteria may induce innate immunity, molecular mimicry, and autoimmunity as well as direct infection of tissues. Several studies suggest a role for *Chlamydia pneumoniae* in atherosclerosis. Not only the ligands but altered receptors have a role in atherogenesis. It was shown that TLRs are expressed in atherosclerotic lesions and may also participate in inflammatory signalling. TLR4 activation by LPS increases atherosclerotic plaque formation in the apoE3*Leiden atherosclerotic mouse model. Human epidemiological data demonstrate that an Asp299Gly TLR4 polymorphism, which attenuates receptor signalling, is associated with a decreased risk of atherosclerosis and acute coronary events. Dual stimulus of acLDL (scavenger receptor priming) and LPS (TLR-4 stimulation) of monocytes may be model for the human coronary events.

In this study, human primary monocyte co-incubation with acLDL increased the amount of IL-8 produced in response to LPS, similar to that seen in monocytic cell lines, raising the possibility that TRB-2 may also be an important negative regulator of IL-8 production of primary monocytes, in response to LPS. These data are in line with previous findings that IL-8 has an important role in atherogenesis. IL-8 *in situ* hybridization experiments using human coronary atherectomy specimens showed that IL-8 mRNA is expressed in a macrophage-rich area of the lesion, consistent with expression in macrophage foam cells. It was also revealed that elevated plasma levels of IL-8 were associated with an increased risk of CAD in apparently healthy individuals.

The experiments in this study give evidence that tribbles, a novel group of proteins, can act as regulators of innate immune responses in monocytes. Tribbles appear to function not only in cell specific, but also in stimulus specific manners. Although from these results it is clear that tribbles action is highly specific; they regulate certain aspects of MAPK action (IL-8 expression) but not others (LDL uptake), within the same cell type. The molecular basis of this phenomenon is currently unknown. Expression of other components of MAPK pathways and/or their state of activation may profoundly influence tribbles action.

Keeshan *et al* showed that retroviral expression of *TRB-2* immortalized hematopoietic progenitors in vitro, and induced fatal transplantable AMLs in murine recipients. Their studies identified TRB-2 as an oncoprotein that contributes to the pathogenesis of AML through the inhibition of C/EBPα function.

Naiki *et al*. found that expression of TRB-2 and TRB-3 was immediately down-regulated in response to differentiation stimuli in 3T3-L1 preadipocyte. While TRB-3 suppressed adipogenesis by strong inhibition of Akt activation, TRB-2 blocked adipogenesis through the inhibition of Akt activation and degradation of C/EBPβ and C/EBPδ.

These results support the observation that tribbles action is highly specific, cell type and stimuli dependent. Tribbles have a role in the regulation of several different signalling pathway (MAPK, Akt, CEBP) thereby they may be essential participants in the development of pathological processes.

Better understanding of the functions and mechanism of tribbles may contribute to comprehension of the regulation of signal processes and thereby some of the pathological disorders.

Publications related to the thesis

I. LDL uptake by monocytes in response to inflammation is MAPK dependent but independent of tribbles protein expression
Katalin Eder, Hongtao Guan, Hye Y. Sung, Sheila E. Francis, David C. Crossman and Endre Kiss-Toth
Immunology Letters, 2008, *In Press* IF: 2,352

II. Human Tribbles-1 controls proliferation and migration of smooth muscle cells via MAPK signalling pathways
H. Y. Sung, H. Guan, A. Czibula, A. R. King, K. Eder, E. Heath, S. K. Suvarna, S. K. Dower, A. G. Wilson, S. E. Francis, D. C. Crossman and E. Kiss-Toth
Journal of Biological Chemistry, 2007, 282(30):21962-72 IF: 5,808

III. Tribbles-2 is a novel regulator of monocyte activation in Coronary Artery Disease

Katalin Eder, Hongtao Guan, Hye Y. Sung, Paul J. Sheridan, Jon Ward, Allison Morton, Adrienn Angyal, Michelle Janas, Gabriella Sarmay, Erno Duda, Martin Turner, Steven K. Dower, Sheila E. Francis, David C. Crossman and Endre Kiss-Toth

Submitted

Other publications

I. Kuppfer cell blockade improves the endotoxin induced microcirculatory inflammatory response in obstructive jaundice

Abraham, Szabolcs; Szabo, Andrea; Kaszaki, Jozsef; Varga, Renata; Eder, Katalin; Duda, Erno ; Lazar, Gyorgy; Tiszlavicz, Laszlo; Boros, Mihaly ; Lazar, Gyorgy Jr

Shock, 2008, *In Press* IF: 3,318

II. Budesonide, but not tacrolimus, affects the immune functions of normal human keratinocytes

K. Kis, L. Bodai, H. Polyanka, K. Eder, A. Pivarcsi, E. Duda, G. Soos, Z. Bata-Csorgo and L. Kemeny

International Immunopharmacology, 2006, 6:358-368 IF: 2,157

Acknowledgments

First of all I would like to express my deep and sincere gratitude to my supervisors *Professor Ernő Duda* (University of Szeged) and *Dr. Endre Kiss-Tóth* (University of Sheffield, United Kingdom) for introducing me to the scientific research. From both professional and human aspects, it was a great pleasure to work under their guidance and to learn so much about immunology. Their wild knowledge, understanding, encouraging and valuable advices served as an excellent basis for the preparation of this thesis. I am grateful to *Dr. Endre Kiss-Tóth* gave me possibility to work at his group in Sheffield.

I express my special thanks to *Dr. Zsuzsanna Győrfy, Erzsébet Kúsz***,** from the Biological Research Center (Szeged), and *Hye Youn Sung, Adrienn Angyal, Jon Ward* Cardiovascular Research Unit (University of Sheffield, UK), and all my other colleagues, who helped me very much as colleagues and as friends, and created a pleasant working environment.

This study has been supported by a short term visiting fellowship of EFIS and by the British Heart Foundation project grants.

I owe a dept of gratitude to my beloved family who taught me persistence and always supported, encouraged me and taught me optimistic thinking.