Abstract

Fibrosis can occur in tissues in response to a variety of stimuli. Following tissue injury, cells undergo transformation or activation from a quiescent to an activated state resulting in tissue remodelling. The fibrogenic process creates a tissue environment that allows inflammatory and matrix-producing cells to invade and proliferate. While this process is important for normal wound healing, chronicity can lead to impaired tissue structure and function.

This review examines the major factors involved in transforming or activating tissues towards fibrosis. The role of genetic variation within individuals affected by fibrosis has not been well described and it is in this context that we have examined the mediators of remodelling, including transforming growth factor-beta, T helper 2 cytokines and matrix metalloproteinases.

Finally we examine the role of Toll-like receptors in fibrosis. The inflammatory phenotype that precedes fibrosis has been associated with Toll-like receptor activation. This is particularly important when considering gastrointestinal and hepatic disease, where inappropriate Toll-like receptor signalling, in response to the local microbe-rich environment, is thought to play an important role.

Background

Fibrosis is a wound-healing response by which the body attempts to repair itself following injury. Acute and, more commonly, chronic injury from a wide variety of insults leads to organ fibrosis. Organ systems have different cellular and molecular mechanisms that result in fibrosis [1-6]. Fibrous tissue contains extracellular matrix (ECM) but in different ratios to normal tissue. In particular, there is a significantly increased amount of type I collagen, with progressive fibrosis eventually leading to a distortion of the normal organ architecture [7,8]. The distorted architecture, along with loss of normal cellularity, leads to a loss of function of the underlying organ. For example, fibrosis in the liver can interfere with drug metabolism, cause accumulation of toxic metabolites and lead to the synthetic failure of important coagulation factors. In lung tissue, fibrosis leads to poor blood-gas exchange resulting in progressive hypoxia as the disease process advances. There is also strong evidence linking fibrotic progression and angiogenesis [9].

Fibrous tissue is laid down by cells with a mesenchymal-like phenotype. In the liver the hepatic stellate cell (HSC) is the major cell responsible for fibrosis, with activation of HSC being a key fibrotic event, although fibroblasts and bone marrow derived fibrocytes all contribute [10-13].
Fibrosis has been described in virtually every organ and most evidently in the liver. Other organs include the lungs, skin, blood vessels, heart and kidneys. In the UK, excessive alcohol consumption remains the most common cause of hepatic fibrosis. Worldwide chronic hepatitis B and C are the principle factors and are a major cause of morbidity and mortality. At a molecular level, transforming growth factor-beta (TGF-β), tissue inhibitor of metalloproteinases (TIMP) and matrix metalloproteinases (MMP) are the key factors involved in the development of fibrogenesis [14,15]. There are a number of other factors contributing to fibrogenesis that may play more significant roles in particular organs. Reactive oxygen species in lungs and liver [16-18], hypoxia inducible factor in kidneys [4] and angiotensin II in blood vessels [19] are examples. In this review we focus on TGF-β, IL-13, TIMP and MMP, the major factors implicated in fibrogenesis.

The role of TGF-β in fibrogenesis

The elucidation of the pathways involved in TGF-β signal transduction has provided new therapeutic targets for the prevention or treatment of fibrosis. TGB-β is a pleiotrophic growth factor which is involved in fibroblast chemotaxis and proliferation. Transient TGF-β1 activity is known to participate in the repair and regeneration of tissues. However, persistent TGB-β1 function induces excessive fibrosis and, ultimately, scarring of both skin and internal organs [20]. TGB-β promotes production of several ECM proteins, including type I collagen, by stimulating its gene transcription. It also influences MMP/TIMP expression and T cell function and, thus, inflammatory reactions are also influenced by TGF-β [21]. COL1A1 and COL1A2 are the genes encoding the polypeptides which form Type I collagen which is the most abundant product of fibrosis, with the development of fibrosis corresponding with an increased rate of the transcription of these two genes [22-24]. Interestingly, enhancer sequences for COL1A1 include binding sites for Smad, Sp1, p38 MAPK and NF-1 [25]. The enhancer region for COL1A2 contains corresponding regions for Smad, Sp1, AP-1 [26-29]. These regulatory gene proteins are known to enhance the effects of TGF-β COL1A2 expression along with the cAMP response element binding protein (CBP) and p300 coactivators [30].

TGF-β is secreted in inactive form which is then activated following proteolysis [21]. Once active, TGF-β is free to bind to its receptors and the resulting signal transduction pathway in the cytoplasm involves activation/translocation of Smad (a family of gene regulatory proteins) to the nucleus (Figure 1). Smad 1, 2, 3, 5 and 8, also known as receptor associated Smads (R-Smads), become phosphorylated when the TGF-β and BMP receptors are activated. Once phosphorylated, these R-Smads dissociate from the receptor and must complex with Smad 4 before they can translocate to the nucleus. Transcription of target genes is then achieved when the phosphorylated Smad complex binds to a specific area of DNA. A number of co-activators (for example, CBP and p300) and transcription factors are also involved in modulating sites of transcription [21]. Smad 6 and 7, unlike other members of the Smad family, prevent phosphorylation and thus activation of these Smad complexes are involved in TGF-β receptor degradation [31].

In addition to activation of the Smad signal transduction pathway, TGF-β activates the mitogen activated protein kinase (MAPK) family (Figure 1) [21]. The TGF-β response is enhanced or inhibited depending on the particular MAPK pathway involved. p38 MAPK and c-Jun N-terminal kinase (JNK) can activate Smad 3 [32,33] and p38 MAPK also strengthens interaction between Smad3 and coactivators [34]. Decreased Type I collagen expression has generally been demonstrated with p38 MAPK inhibition [32,33,35]. The final MAPK pathways - extracellular signal regulated kinase (ERK) - inhibit Smad signal transduction, as well as BMP, and Smad1 effects on transcription [36]. The impact of ERK on collagen gene transcription is cell specific with ERK causing increased collagen production in some cells and decreased production in others [37].

IL-13 and fibrosis

The cytokine environment also plays a role in tissue remodelling and it seems likely that they can influence the phenotypic changes seen in different cell types in fibrotic tissue. IL-13 is a Th2 cytokine that is known to induce fibrosis through the regulation of TGF-β1 production and activation [38,39]. IL-13 binds to two primary receptor chains IL-13Ralpha1 and IL-13Ralpha2 (Figure 1). IL-13Ralpha1 is expressed in healthy tissue and binds IL-13 through the formation of a heterodimer complex with IL-4Ralpha chain. This complex formation culminates in signal transduction via the JAK/STAT6 pathway [40,41]. IL-13Ralpha2, on the other hand, is only marginally expressed in normal healthy tissue and over-expressed in several abnormal cells including cancerous and fibrotic. However, it can bind IL-13 with a high affinity with mediation of signal transduction, thought to be STAT6 independent, signalling instead through the AP-1 pathway (Figure 1) [39]. Due to its lack of expression in normal tissue and over expression in cancer cells and during fibrosis, it has been suggested that IL-13Ralpha2 chain may serve as a novel biomarker for diseased cells and a target for receptor-directed therapeutics [39,42,43].

Fibroblast IL-13Ralpha2 expression has been reported in several fibrotic diseases including idiopathic interstitial pneumonia, schistosomiasis and non-alcoholic steatohepatitis [44-46]. Within the context of hepatic disease,
IL-13Ralpha2 is expressed in activated HSCs but not quiescent HSCs, with expression strongly induced by both TGF-β1 and also TNF-α [46]. Interestingly, TGF-β independent IL-13 induced fibrosis has also been identified, within the context of parasitic disease, with IL-13Ralpha2 over expression acting as a soluble decoy receptor and ultimately decreasing fibrosis [47].

MMPs and TIMPs

MMPs and TIMPs are also responsible for maintaining integrity of the ECM. There are many different types of metalloproteinases, some of which are specific and others that are less discriminating to ECM substrates. Their presence is required to degrade the wide variety of components of the ECM. Excessive catabolism of ECM is kept in check with several mechanisms, including the secretion of TIMPs. TIMPs work by binding to MMPs thereby blocking their activity.

Expression of MMP subtype is tissue dependent with differences in amino acid sequence of MMPs seen amongst animal species [48]. MMP-1 is significantly expressed by several types of cells including the HSC. MMP-13 is the rodent homologue of human MMP-1 [49-51]. Following liver injury, elevated levels of MMP-1 and MMP-13 RNA have found in human and rodent liver tissue respectively [51-54]. Elevated metalloproteinase expression, in acute liver injury has been shown to occur for only a short period post insult [54] and, in chronic liver injury, is limited to the period of fibrogenesis [55]. After liver injury, HSCs become activated and express MMP-2 and MMP-14 [50,56]. Elevated MMP-2 and MMP-14 levels have been demonstrated in fibrotic and cirrhotic liver tissue, with the exception of HCV induced cirrhosis [57,58]. MMP-2 promotes proliferation and migration of the hepatic stellate cells and its activation is dependent on MMP-14 [59]. Both TIMP-1 and TIMP-2 can inhibit MMP 2.

There is brief expression of MMP-3 following HSC activation and acute toxic liver injury. The most important role MMP-3 is known to play in fibrosis is through cleavage of MMP precursors such as MMP-1, -3, -7, -8, -9 and -13 to their active forms [60-65]. However, there is contradictory evidence in the literature on whether MMP-3 expression is increased or decreased in chronic liver injury [66-68]. Similarly, variable results have been obtained for MMP-9 expression in both acute and chronic liver injury [57,69-71]. IL-13 is a potent inducer of MMP-9. The role of MMP-9 in fibrogenesis is thought to primarily involve activation of TGF-β [72]. This is significant as initial collagen production by the HSC is stimulated by TGF-β [73].

TIMP-1 and TIMP-2 are the main TIMPs associated with fibrosis. In the liver, these TIMPs are primarily produced by HSCs, although other cells also contribute to TIMP production [74]. In hepatitis C infection, the degree of hepatic fibrosis is correlated with the level of TIMP-1 mRNA and protein [53]. In addition to binding, and thereby inhibiting matrix metalloproteinases, TIMP-1 also prevents apoptosis of HSCs [75,76]. Elevated levels of TIMP-2 in serum and liver mRNA are found in human hepatitis C virus (HCV) liver disease. However, fibrosis does not have to be present for raised TIMP-2 levels to be detectable in HCV patients [77,78]. In rodents, TIMP-2 mRNA reaches its highest levels in acute toxicity within 3 days, whereas it is not increased with chronic toxic liver injury [57,70,79].

Host genetic factors and fibrosis

Before discussing the role of host genetic factors in fibrosis, it is essential to establish some basic principles of genetic epidemiology and the limitations of studying genetic polymorphisms in the context of complex multifactorial human diseases [80]. The overwhelming major-
ity of polymorphisms studied are single nucleotide polymorphisms (SNPs) that occur with a frequency of >1% in the normal population (in contrast to ‘mutations’ that occur with a frequency of <1%). It is estimated that the human genome contains up to 10 million SNPs, although not all have thus far been identified. Most SNPs are located within non-coding regions of the genome. However, of those that are located within coding sequence, most are non-synonymous and are not associated with the alteration of the amino acid sequence rendering them of no functional consequence. Other types of genetic variation include deletion and insertion polymorphisms and microsatellite repeat polymorphisms.

There has been an exponential rise in the number of published genetic association studies. Quite often, a report of a single genetic marker is published with great promise, only to be followed by several negative studies that fail to reproduce the original observation. There is no doubt that the strategy of genetic association studies could be a powerful tool for dissecting human diseases, provided certain principles are observed in order to minimize the chances of false positive, and negative, reports. The most important of these principles include: rigorous definition of disease phenotype; choice of candidate genes that are plausibly linked to the pathophysiology of the disease under study; selection of polymorphisms with known (or at least potentially) functional consequences; choice of genetic markers that are reasonably frequent in the population under study (variant allele frequency of at least 5%); appropriate selection of controls that are matched for ethnicity, age, gender and environmental exposures; and design of studies that are adequately powered to produce a valid result. Even then, the statistical analyses of such studies have to take into account the real problem of false positive results by using multiple testing. Appropriate corrections for multiple testing have to be applied or, alternatively, the positive findings should be regarded as preliminary and should be validated in an independent set of cases and controls. Finally, the genetic epidemiology has to involve basic science in order to unravel and validate the molecular mechanisms involved. Adherence to these basic principles will ensure that false positive trails are minimized and will offer a true opportunity to understand the complex multifactorial human diseases. There has never been a better time to stress the necessity for an adherence to these principles, as the advancement in genotyping technology has made possible the annotation of the entire human genome. Current technology allows us to genotype up to 500,000 SNPs in one run. We have witnessed a shift towards these so-called whole genome association studies, where large multi-centre consortia attempt to examine a very large number of cases and controls for a particular disease. The power of these studies allows for an exploratory phase where thousands of SNPs are examined and a validation phase that attempts to replicate positive associations independently.

Having set the background to the study of genetic polymorphisms, we can now examine the role of these in the context of fibrosis. Pathogenic fibrosis typically results from chronic inflammatory reactions, many of which will be triggered by an infectious agent or a chemical assault which drives the chronic inflammation and the subsequent development of fibrosis. The role of polymorphisms in several cytokine genes has been examined in the context of fibrotic disease, often with conflicting results. We will concentrate on genetic markers that have relevance to pathogenesis of fibrotic diseases and will only consider markers that satisfy the criteria listed above.

Perhaps the most relevant gene in the context of fibrosis is TGF-β. Several SNPs have been identified in this gene and some are associated with elevated TGF-β1 concentrations in human plasma [81-83]. However, only SNPs within the coding region of TGF-β1 (Leu10Pro and Arg25Pro) have been shown to be associated with increased fibrotic risk [84-88] (Table 1). Gewaltig et al. reported that the carriage of at least one Pro at codons 10 and/or 25 was significantly associated with a faster progression of hepatic fibrosis following chronic hepatitis C infection. The fibrosis progression rate of patients with genotypes 10LeuPro and 10ProPro was almost three times as fast as those having genotype 10LeuLeu. Stage and histological activity grade of fibrosis in 25ArgPro in comparison to 25ArgArg were also higher [84]. Tag et al. were able to reproduce similar findings reporting an increased risk of higher grades of fibrosis in carriers of the 25ArgPro genotype [85]. However, these were small studies and findings from other groups have either failed to replicate the associations or reported opposite associations. For example, Powell et al. showed that the 25ArgArg genotype was associated with increased risk of hepatic fibrosis following HCV infection [87]. The same polymorphisms have been addressed in other hepatic disorders. Osterreicher et al. studied the role of host genetic factors in the progression of hereditary haemochromatosis and showed that the 25ArgPro genotype increased the risk of cirrhosis by nearly threefold compared to 25ArgArg genotype [86]. The direction of association is similar to that reported by Gewaltig and Tag et al. but the studies remain small and require definitive validation in larger case control studies.

TGF-β1 production is also known to be enhanced by angiotensin II, the principal effector molecule of the renin-angiotensin system. A statistically significant relationship was also seen between the polymorphism in the promoter region of the angiotensinogen gene (A17-6) and the stage of hepatic fibrosis [87]. Individuals with the adenine/adenine homozygous genotype were more likely to have
increased hepatic fibrosis compared with individuals inheriting the adenine/guanine or the guanine/guanine homozygous genotype (Table 1).

The TNF-A-308 G > A polymorphism is known to be involved in a number of inflammatory conditions. Carriage of the pro-inflammatory A allele has been shown to increase the odds ratio for severe disease in both hepatic fibrosis and also fibrosing alveolitis (Table 1). Yee et al. reported carriage of the -308A allele was associated with a fivefold increased risk of cirrhosis following HCV infection [89]. These findings were reported by Kusumoto et al., with carriage of ‘A’ at TNF-α -238 or -308 correlating with significantly higher serum levels of Type IV collagen 7S, which is a marker for advanced hepatic fibrosis [90]. However, other reports failed to confirm these associations. Carriage of TNF-A-308 A has also been assessed within the context of fibrosing alveolitis in several small studies. Whyte et al. assessed the frequency of the polymorphism in two independent case-control studies, one English and one Italian, and showed a significant association of TNF-A-308 A carriage with increased risk of fibrosing alveolitis in the Italian, but not the English, study [91]. Studies by Pantelidis et al. and Riha et al. confirmed this association but the findings require confirmation in a much larger study with appropriately matched controls [92,93].

Other cytokine genes in which genetic variation has been examined within the context of fibrotic disease include interleukin-10, interferon-gamma and the interleukin (IL)-1 receptor antagonist. IL-10 is an anti-inflammatory Th2 cytokine that down regulates IL-1β, TNF-α, interferon-γ and other pro-inflammatory cytokines and has a modulatory effect on hepatic fibrogenesis. IL-10 levels differ widely between individuals, possibly because of polymorphisms in the promoter region of the IL-10 gene at positions -592, -819 and -1082 [94,95]. Promoter polymorphisms have been associated with several inflammatory conditions including hepatitis B virus-induced hepatocellular carcinoma and other cancers [96,97]. We have previously reported that homozygosity for the low-IL-10 ATA haplotype increased the risk of non-cardia gastric cancer [98]. IL-10 SNPs have been studied in the context of hepatic fibrosis. An early study by Powell et al. did not show a correlation between stages of HCV induced fibrosis and IL-10 promoter polymorphisms [87]. However, a study published the same year, looking at their role in alcoholic liver disease induced fibrosis, indicated a strong association between possession of the A allele at position -592 in the IL-10 promoter region and fibrosis [99]. It was subsequently suggested that defining disease progression would possibly be more appropriate based on the speed of fibrotic development (that is, fast versus slow). A subsequent study by Knapp et al. looking at HCV-induced fibrosis, showed a higher frequency of the low IL-10 producing haplotypes (ACC/ACC and ATA/ATA) in patients termed ‘fast fibrosores’ [100] (Table 1).

Polymorphisms in interleukin-1 that have been assessed in the context of fibrotic disease are mainly related to the IL-1 receptor antagonist. Whyte and colleagues looked at the IL-1RN polymorphism at C+2018T in two European cohort studies of pulmonary fibrosis along with the previously mentioned TNF-A-308 G > A polymorphism [91]. As with the TNF-A-308 G > A polymorphism, carriage of the rarer T allele at IL-1RN +2018 was associated with an increased risk of fibrosing alveolitis in the Italian but not the English cohort. This is again possibly due to small study numbers. A variable tandem repeat genetic variant in intron 2 of IL-1RN which is in strong linkage disequilibrium with the C+2018T has also been studied. However, no association has been defined in either hepatic or pulmonary fibrosis [93,101,102]. Carriers of interferon-gamma (IFN)-G +874 T allele have also been shown to have a significantly higher rate of liver cirrhosis and early recurrent hepatitis C after transplantation [103,104]. The AA genotype is associated with the low levels of IFN-gamma production which is thought to inhibit the appropriate level of T-helper (Th1) response needed to combat HCV viral load and subsequent disease progression [105,106].

Assessment of genetic variation in chemokine receptors has also been studied in the context of HCV-induced hepatic fibrosis. Hellier et al. reported a significant association between severe HCV-induced hepatic fibrosis and carriage of both CCR5 Δ32 and the K variant of the MCP-2 Q46K polymorphism [107]. In total 20 polymorphisms in seven CC chemokines and their receptors were assessed in the study which comprised 672 patients. Both chemokines are involved in T cell recruitment/migration and processes relevant to HCV clearance or persistence. Particularly in relation to CCR5 Δ32, for which functional data is available, carriage of the 32 bp deletion results in a non-functional protein which will impact on viral persistence [108]. The -2518 MCP-1 promoter polymorphism has also been shown to be a risk factor for HCV induced hepatic fibrosis. An elegant study by Muhlbauer et al. showed that carriage of MCP-1 -2518 G allele, which is associated with increased MCP-1 levels, was associated with more advanced fibrosis and severe inflammation [109]. The study also demonstrated, for the first time, an association of the MCP-1 polymorphism with MCP-1 tissue levels.

Polymorphisms involved in other cellular processes important in hepatic fibrotic development have also been studied. Mutations within the haemochromatosis (HFE) gene involved in iron storage and accumulation have been shown to be associated with higher grades of inflam-
Table 1: Genetic polymorphisms with relevance to fibrosis risk.

| Gene                        | Known variation | Effect                                                                 | Reference                  |
|-----------------------------|-----------------|------------------------------------------------------------------------|----------------------------|
| **TGF beta**                |                 |                                                                        |                            |
|                             | Leu10Pro        | LeuLeu showed a slow progression of fibrosis Carriage of Pro associated with faster fibrotic progression | [84-88]                    |
|                             | Arg25Pro        | Carriage of Pro associated with faster fibrotic progression            |                            |
| Angiotensin                 | G-6A            | Carriage of AA genotype associated with increased risk of fibrosis    | [87]                       |
| **TNF-alpha**               | G-308A          | A allele associated with hepatic fibrosis, hepatic cancer, fibrosing alveolitis | [91,93,136,137]            |
| **IL-10**                   | C-592A/-819/G-1082A | Carriage of ATA associated with ALD and HCV induced fibrosis          | [99,100,138]               |
| **IL-1**                    | IL-1RNC +2018T  | T allele associated with increased risk of fibrosing alveolitis       | [91]                       |
| **IFN-gamma**               | T+874A          | T allele associated with higher rate of liver cirrhosis following Hep C infection | [103,104]                  |
| **CC chemokine receptor 5 (CCR5)** | insertion/deletion (Δ32) | Carriage of Δ32 associated with severe fibrosis | [107]                     |
| **MCP-2**                   | Q46K            | Carriage of the K variant is associated with more severe fibrosis     | [107]                      |
| **MCP-1**                   | G-2518A         | Carriage of G allele associated with increased risk of hepatic inflammation and fibrosis | [109]                      |
| **Haemochromatosis gene (HFE)** | G+845A C+187G  | Heterozygous genotypes associated with increased inflammation and fibrosis | [110,111]                 |
| **Myeloperoxidase (MPO)**   | G-463A          | Minor allele associated with increased risk of advanced fibrosis in CHC patients | [112]                      |
| **low density lipoprotein receptor (LDLR)** | G+1170A | Carriage of G associated with increased risk of fibrosis | [113]                     |
| **Apolipoprotein E (Apo E)** | E4 allele       | Carriage of E4 allele associated with protection against HCV induced liver damage | [114]                      |
| **DDX5 DEAD box polypeptide 5 and POLG2 SNPs** |                 | Minor allele associated with increased risk of advanced fibrosis in CHC patients | [139]                      |
| **CD14**                    | C-159T          | T allele associated with higher levels of acute phase proteins and advanced ALD | [140]                      |
| **TLR4**                    | D299G T399I     | Both variant allele confer protection against fibrosis                | [141,142]                  |

CHC, chronic hepatitis cancer; ALC, alcoholic liver cirrhosis; ALD, alcoholic liver disease; HCV, hepatitis C virus

mation and more severe hepatic fibrosis, although these findings were not replicated in other published studies [110,111]. Potential explanations for the lack of validation include: small sample size; different histological scores for hepatic fibrosis; ethnicity; population stratification; and uncontrolled variables associated with disease progression. A promoter polymorphism within the myelo-peroxidase gene which is involved in activation of HSCs and the production of ECM-MPO G-463A has also been shown to be associated with advanced fibrosis when the variant A allele was present [112]. Polymorphisms in genes involved in lipid transportation have also been
assessed within the context of HCV-induced liver fibrosis. These pathways are thought to promote viral endocytosis. Carriage of the G allele of the low density lipoprotein receptor polymorphism - G+1170A - has been shown to render patients more susceptible to developing severe HCV-induced fibrosis [113]. Conversely, carriage of the apolipoprotein (apoE) E4 allele has been shown to protect against severe liver damage induced by HCV [114] (Table 1).

**Toll-like receptors (TLRs) and fibrosis**

There is growing interest in the role of the innate immune system, especially TLRs, as regulators of wound healing and especially fibrosis. TLRs are a highly conserved family of germline-encoded receptors that recognize structural motifs expressed by bacteria, viruses and fungi. Stimulation of TLRs by these ligands activates numerous signalling cascades which ultimately culminate in proinflammatory cytokine production and other immune responses, including cell survival and apoptosis [115]. Currently 10 human TLRs have been identified, each with different ligand specificity. TLR4 is known as the lipopolysaccharide (LPS) receptor, due to the original reports which demonstrated the relationship between TLR4 and LPS recognition [116,117]. LPS - or endotoxin - a component of the Gram-negative bacterium outer membrane is are now known to be one of a collection of ligands that is recognized by TLR4. However, it is known that TLR4 (and possibly other TLRs) can detect other exogenous as well as endogenous ligands, many of which are most abundant during tissue injury such as hyaluronan, fibronectin S100 proteins and heat shock proteins 60 and 70 [118]. Along with TLR4, TLRs 1, 2, 5, 6 and 9 are involved in bacterial recognition. TLR 1, 2 and 6 recognize lipoprotein from Gram-positive bacteria and TLR5 is involved in bacterial flagellin sensing. TLR9 recognizes non-methylated CpG-containing DNA from bacteria. In contrast, TLR 3, 7, 8 and 9 recognize viral nucleic acids. TLRs although similar in their structure with a leucine-rich repeat domain and a Toll/IL-1 receptor (TIR) domain are separated on the basis of their cellular location with TLR 1, 2, 4, 5 and 6 located on the cell surface, whilst the others are associated with endosomal/lysosomal compartments where the possibility of encountering host DNA and therefore eliciting self-recognition is reduced [119].

Following ligand binding, TLR signalling cascades are initiated from the TIR domain and many of the signalling molecules that mediate the intracellular response are common between the TLRs [120]. TLR signalling has been divided into MyD88-dependent and MyD88-independent (TRIF dependent) pathways (Figure 2). MyD88-dependent signalling culminates in activation of the transcription factors NF-kB and AP-1 (via downstream MAPK pathways) and the production of numerous pro-inflammatory cytokines and immune mediators. These transcription factors are also activated via MyD88-independent signalling but their activation is slightly delayed [121]. All TLRs with the exception of TLR3 signal via the MyD88-dependent signalling pathway. MyD88-independent signalling is involved in the induction of interferon-inducible genes including IRF3 which are important for anti-viral and anti-bacterial responses [122,123]. TLR4 is the only TLR known to utilize both the MyD88-dependent and independent pathways [124,125].

Although all immune cells express TLRs, these receptors are also present on other classes of cell. Nevertheless, the ability of different cell types to recognize and respond to microbial ligands differs. Generally, TLR expression on immune cells is there as the archetypal response to infection. TLR activation on other cell types, including epithelial cells, whilst contributing to the immune response has also been suggested to lead to tissue scarring and fibrosis [126,127].

Impaired TLR4 and nine responses through defective signalling, and also the presence of genetic variations, have been shown to reduce hepatic fibrosis [128-130]. A number of *in vivo* observations also support a role for TLRs in promoting fibrogenesis, although this has only been studied within the context of hepatic disease. It has also been shown that the intestinal microbiota is at least in part responsible for activating TLR4 containing cells within the liver, especially quiescent HSCs ultimately evoking hepatic fibrogenesis through modulation of TGF-β signalling [128,131-133]. TLR induced activation of p38 MAPK and JNK has also been shown to be involved in increased production of collagen by HSCs [134]. The link between the intestinal microbiota and hepatic TLR activation is through the portal vein. The most likely explanation is that damage incurred to the portal system, during chronic hepatic injury, affects the intestinal barrier function allowing increased bacterial translocation [128,129] (Figure 2). This view is supported by studies using gut-sterilized mice that have shown a strong reduction in fibrogenesis compared to conventional mice [135].

**Conclusion**

Fibrosis can occur in almost any tissue type, with analysis of the cellular and molecular mechanisms showing similarities irrespective of location. Trauma/insult usually through exogenous stimuli, chemical or microbiological, results in innate immune cell activation which triggers a chronic inflammatory response that is central to fibrotic perpetuation. TGF-β plays a pivotal role in the fibrotic development through its influence on MMP/TIMP expression, T cell function and also ECM production. However,
further studies are required in order to fully understand the complex relationship. The situation is further complicated by the contribution of host genetic polymorphisms to an individual's risk.

Chronic inflammation, whether caused by microbes, chemical or physical trauma favours fibrotic progression. A series of intricate host responses are initiated that, on one hand, are attempting to initiate repair of the tissue damage through resolution of the inflammation, whilst also trying to eliminate the infection. The Th2-type cytokine response (typified by IL-13) seen in fibrosis is pivotal to this as is the increasing understanding of TLR signalling and the impact of genetic polymorphisms on these systems. Therapies aimed at suppressing TLR4 signalling, either through preventing LPS release or TLR4 inhibition, is already being considered in the context of hepatic fibrosis, although other fibrotic targets are also under investigation. Understanding the interplay between trauma stimuli and tissue repair is fundamental to resolving the complex interplay between the causes of chronic inflammation and the host's genetic disposition to fibrotic progression, which will aid the development of new and more effective anti-fibrotic strategies in the future.
Abbreviations

- CBP: cAMP response element binding protein
- ECM: extracellular matrix
- ERK: extracellular signal related kinase
- HCV: hepatitis C virus
- HFE: haemochromatosis
- HSC: hepatic stellate cell
- IFN: interferon
- IL: interleukin
- JNK: c-Jun N-terminal kinase
- LPS: lipopolysaccharide
- MAPK: mitogen activated protein kinase
- MMP: matrix metalloproteinase
- R-Smad: receptor associated Smad
- SNP: single nucleotide polymorphism
- TGF: transforming growth factor
- TIMP: tissue inhibitor of metalloproteinase
- TIR: Toll/IL-1 receptor
- TLR: Toll-like receptor

Competing interests

The authors declare that they have no competing interests.

References

1. Maher JJ, McGuire RF: Extracellular matrix gene expression increases preferentially in rat lipocytes and sinusoidal endothelial cells during hepatic fibrosis in vivo. J Clin Invest 1990, 86(5):1641-1648.
2. Weiner FR, Giambone MA, Czaja MJ, Shah A, Annoni G, Takahashi S, Egahlmi M, Zern MA: Ito-cell gene expression and collagen regulation. Hepatology 1990, 11(1):111-117.
3. Kawaguchi Y, Suzuki K, Hara M, Hidaka T, Ishizuka T, Kawagoe M, Nakamura H: Increased endothelin-1 production in fibroblasts derived from patients with systemic sclerosis. Ann Rheum Dis 1994, 53(8):506-510.
4. Abraham DJ, Chambers RC: Molecular targets in pulmonary fibrosis: the myofibroblast in focus. Chest 2007, 132(4):1311-1321.
5. Higgins DF, Kinura K, Iwano M, Haase VH: Hypoxia-inducible factor signaling in the development of tissue fibrosis. Cell Cycle 2008, 7(9):1128-1132.
6. Abraham DJ, Yancheewaran R, Dashwood RM, Black CM: Increased expression of endothelin-1 and differential endothelin type A and B receptor expression in sclerodema-associated fibrotic lung disease. Am J Pathol 1997, 151(3):831-841.
7. Milani S, Herbst H, Schuppan D, Kim KY, Riecken EO, Stein H: Procollagen expression by nonparenchymal rat liver cells in experimental biliary fibrosis. Gastroenterology 1990, 98(1):175-184.
8. Maher JJ, Bissell DM, Friedman SL, Roll FJ: Collagen measured in primary cultures of normal rat hepatocytes derives from lipocytes within the monolayer. J Clin Invest 1988, 82(3):450-459.
9. Parola M, Marra F, Pinzani M: Myofibroblast-like cells and liver fibrogenesis: Emerging concepts in a rapidly moving scenario. Mol Aspects Med 2008, 29(1-2):58-66.
10. Bataller R, Paik YH, Lindquist JN, Lemasters JJ, Brenner DA: Hepatitis C virus core and nonstructural proteins induce fibrogenic effects in hepatic stellate cells. Gastroenterology 2004, 126(2):529-540.
11. De Minicis S, Seki E, Uchimani H, Kluwe J, Zhang Y, Brenner DA, Schwabe RF: Gene expression profiles during hepatic stellate cell activation in culture and in vivo. Gastroenterology 2007, 132(5):1937-1946.
12. Moore BB, Kolodisch JE, Thinnickal VJ, Cooke K, Moore TA, Hoagboam C, Wllke CA, Toews GB: CCR2-mediated recruitment of fibrocytes to the alveolar space after fibrotic injury. Am J Pathol 2005, 166(4):675-694.
13. Kisseleva T, Uchimani H, Feirt N, Quintana-Bustamante O, Segovia JC, Schwabe RF, Brenner DA: Bone marrow-derived fibrocytes participate in pathogenesis of liver fibrosis. J Hepatol 2006, 45(3):429-438.
14. Gauldie J, Bonniaud P, Sime P, Ask K, Kolb M: TGF-beta, Smad3 and the process of progressive fibrosis. Biochem Soc Trans 2007, 35(Pt 4):661-664.
15. Leask A: Targeting the TGFbeta, endothelin-1 and CCN2 axis to combat fibrosis in scleroderma. Cell Signal 2008, 20(8):1409-1414.
16. Porter DW, Milleccia LL, Willard P, Robinson VA, Ramsey D, McLaurin J, Khan A, Brumbaugh K, Beighley CM, Teass A, Castranova V: Nitric oxide and reactive oxygen species production causes progressive damage in rats after cessation of silica inhalation. Toxicol Sci 2006, 90(1):188-197.
17. Parola M, Robino G: Oxidative stress-related molecules and liver fibrosis. J Hepatol 2001, 35(2):297-306.
18. Jaeschke H: Mechanisms of liver injury. II. Mechanisms of neutrophil-induced liver cell injury during hepatic ischemia-reperfusion and other acute inflammatory conditions. Am J Physiol - Gastrointestinal Liver Physiol 2006, 290(6):G1083-G1088.
19. Ruiz-Ortega M, Ruperez M, Esteban V, Egido J: Molecular mechanisms of angiotensin II-induced vascular injury. Curr Hypertens Rep 2003, 5(1):73-79.
20. Carverone KMS: TGF-beta-induced fibrosis and SMAD signaling: oligo decoys as natural therapeutics for inhibition of tissue fibrosis and scarring. Wound Repair Regen 2007, 15:S54-S60.
21. Inagaki Y, Okazaki I: Emerging insights into transforming growth factor β1 Smad signal in hepatic fibrogenesis. Gut 2007, 56(2):284-292.
22. Verrecchia F, Maivial A: TGF-β and TNF-α antagonistic cytokines controlling type I collagen gene expression. Cell Signal 2004, 16(8):873-880.
23. Ghosh AK: Factors involved in type I collagen gene expression: implication in scleroderma. Exp Biol Med 2002, 227:301-314.
24. Trojanowska M, Carwile LeRoy E, Eckes B, Krieg T: Pathogenesis of fibrosis: type I collagen and the skin. J Mol Med 1998, 76(3):266-274.
25. Jimenez SA, Varga J, Olsen A, Li D, Diaz A, Herijal H, Koch J: Functional analog of human α1 (I) procollagen gene promoter: differential activity in collagen producing and nonproducing cells and response to transforming growth factor β1. J Biol Chem 1994, 269(12):12684-12691.
26. Chung KY, Agarwal U, Uitto J, Maivial A: An AP-1 binding sequence is essential for regulation of the human alpha 2 (I) collagen (COL1A2) promoter activity by transforming growth factor β1. J Biol Chem 1996, 271:3272-3278.
27. Verrecchia F, Rossert J, Maivial A: Blocking Sp1 transcription factor broadly inhibits extracellular matrix gene expression in vitro and in vivo: implications for the treatment of tissue fibrosis. J Invest Dermatol 2001, 116(5):755-763.
28. Chen SJ, Yuan W, Mori Y, Varga J: Stimulation of type I collagen transcription in human skin fibroblasts by TGF-b: involvement of Smad 3. J Invest Dermatol 1999, 112:49-57.
29. Poncelet AC, Schnaper HW: Sp1 and Smad proteins cooperate to mediate transforming growth factor-β1-induced α2(I) collagen expression in human glomerular mesangial cells. J Biol Chem 2001, 276(10):6983-6992.
30. Greenwell P, Inagaki Y, Hu W, Walsh M, Ramirez F: Sp1 is required for the early response of alpha 2 (I) collagen to transforming growth factor-beta 1. J Biol Chem 1997, 272:19738-19745.
31. Shi Y, Massague J: Mechanisms of TGF-beta1 signaling from cell membrane to the nucleus. Cell 2003, 113(6):685-700.
32. Furukawa F, Matsuzaki K, Mori S, Tahashi Y, Yoshida K, Sugano Y, Yamagata H, Matsushita M, Seki T, Inagaki Y: p38 MAPK mediates fibrogenic signal through Smad3 phosphorylation in rat myofibroblasts. Hepatology 2003, 38(4):879-889.
33. Yoshida K, Matsuzaki K, Mori S, Tahashi Y, Yamagata H, Furukawa F, Seki T, Nishiizawa M, Fujisawa J, Okazaki K: Transforming growth factor-β1 and platelet-derived growth factor signal via c-Jun N-terminal kinase-dependent Smad2/3 phosphorylation in rat hepatic stellate cells after acute liver injury. Am J Pathol 2005, 166(4):1029-1039.
34. Abecassis L, Rogier E, Vazquez A, Afifi A, Bourgeade MF: Evidence for a role of MSK1 in transforming growth factor-β1-mediated responses through p38 (alpha) and Smad signaling pathways. J Biol Chem 2004, 279(29):30474-30479.
50. Knittel T, Mehde M, Kobold D, Saile B, Dinter C, Ramadori G, Lichtinghagen R, Bahr MJ, Wehmeier M, Michels D, Haberkorn CI, Schuppan D, Ruehl M, Somasundaram R, Hahn EGMD.

51. A mechanism of repression of TGF-

52. mRNA expression is enhanced relative to interstitial fibrosis in chronic liver disease. J Hepatol 1999, 30(3):176-184.

53. Expression of matrix metalloproteinase-13 and tissue inhibitor of metalloproteinase-1 in acute liver injury. J Hepatol 1999, 30(3):424-428.

54. Watanabe T, Nikola M, Hozawa S, Kameyama K, Hayashi T, Ari M, Ishikawa A, Maruyama O, Okazaki I. Gene expression of interstitial collagenase in both progressive and recovery phase of rat liver fibrosis induced by carbon tetrachloride. J Hepatol 2000, 33(2):224-235.

55. Ikeda K, Wakahara T, Wang YQ, Kadoya H, Kawada N, Kaneda K. In vitro migratory potential of rat quiescent hepatic stellate cells and its augmentation by cell activation. Hepatology 1999, 29(6):1760-1767.

56. Zhou Y, Hoffel CJ, Pawley S, Hutchings MJ, Arbour MP, Iredale JP. Benyon RC. Expression of matrix metalloproteinase-2 and -14 persists during early resolution of experimental liver fibrosis and might contribute to fibrolysis. Liver Int 2004, 24(5):492-501.

57. Takahara T, Furui K, Yata Y, Jin B, Zhang LP, Nambu S, Sato H, Seki M, Watanabe A. Dual expression of matrix metalloproteinase-2 and membrane-type 1 matrix metalloproteinase in fibrotic human livers. Hepatology 1999, 26(6):1521-1529.

58. Strongin AY, Collier I, Bannikov G, Flamer BL, Grant GA, Goldberg GI. Mechanism of cell surface activation of 73-kDa type IV collagenase. J Biol Chem 1991, 266(52):331-338.

59. Suzuki K, Enghild JJ, Morodomi T, Salvesen G, Nagase H. Mechanisms of activation of tissue procollagenase by matrix metalloproteinase 3 (stromelysin). Biochemistry (N Y) 1990, 29(44):10261-10270.

60. Nagase H, Enghild JJ, Suzuki K, Salvesen G. Stepwise activation mechanisms of the precursor of matrix metalloproteinase 3 (stromelysin) by proteases and 4-aminophenyl mercuric acetate. Biochemistry (N Y) 1990, 29(24):5738-5739.

61. Imai K, Yokohama Y, Nakashima I, Ohuchi E, Fuji Y, Nakai N, Okada Y. Matrix metalloproteinase 7 (MMP-7) from human rectal carcinoma cells: Activation of the precursor interaction with other matrix metalloproteinases and enzymatic properties. Biochim Biophys Acta 1995, 1283-1297.

62. Kossakowska AE, Edwards DR, Lee SS, Urbanski LS, Stabbler AL, et al. Expression of MMPs and TIMPs in liver fibrosis - a systematic review with special emphasis on anti-fibrotic strategies. J Hepatology 2007, 46(5):973-975.

63. Clemens ER, Wehrly KD, Mohler ER, Hoogenboom SA. Spatial and temporal distribution of tissue inhibitor of metalloproteinase-1 mRNA in chronic liver disease. J Hepatol 1999, 30(3):425-432.
Fibrogenesis & Tissue Repair 2009, 2:6
http://www.fibrogenesis.com/content/2/1/6

73. Cao Q, Mak K, Lieber CS: Dihydroxyphosphatidylcholine (DLPC) decreases transforming growth factor-β mediated collagen production by rat hepatic stellate cells. J Lab Clin Med 2002, 139:202-210.

74. Bataller R, Brenner D: Liver fibrosis. J Clin Invest 2005, 115:209-218.

75. Yoshii H, Kuriyama S, Miyamoto Y, Thorgersson US, Gomez DE, Kowata M, Yoshii J, Ikenna Y, Nagase H, Tissue inhibitor of metalloproteinases-1 promotes liver fibrosis development in a transgenic mouse model. Hepatology 2000, 32(6):1248-1254.

76. Murphy FR, Isa R, Zhou X, Ratnarajah S, Nagase H, Arthur MJP, Benyon C, Iredale JP: Inhibition of apoptosis of activated hepatic stellate cells by tissue inhibitor of metalloproteinase-I is mediated via effects on matrix metalloproteinase inhibition implications for reversibility of liver fibrosis. J Biol Chem 2002, 277(13):1069-11076.

77. Bahr MJ, el Menuawy M, Boeker KHW, Musholt PB, Manns MP, Lich Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe Yee LJ, Tang J, Herrera J, Kaslow RA, van Leeuwen DJ: Analysis of tumor necrosis factor-α, lymphotixin-α, tumor necrosis factor receptor II and interleukin-6 polymorphisms in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2001, 163:1432-1436.

78. Riha RL, Yang IA, Rabnott GC, Tunncliffe AM, Feng KM, Zimmerman PV: Cytokine gene polymorphisms in idiopathic pulmonary fibrosis. Intern Med J 2004, 34(6):126-129.

79. Eskdale J, Keijers V, Huizinga T, Gallagher G: Microsatellite alleles and single nucleotide polymorphisms (SNP) combine to form four major haplotype families at the human interleukin-10 (IL-10) locus. Genes Immun 1999, 1(2):151-155.

80. Knapp S, Hennig BJW, Frodsham AJ, Zhang L, Hellier S, Wright M, Goldin R, Hill AVS, Thomas HC, Thursz MR: Interleukin-10 promoter polymorphisms and the outcome of hepatitis C virus infection with hepatocellular carcinoma in Taiwan. Tissue Antigens 2006, 67(2):127-133.

81. Michaud DS, Daughey SE, Berndt SI, Platz EA, Yeager M, Crawford j Emerson J, McLaughlin J, Chen DS, Chen PJ: Correlation of interleukin-10 gene haplotype with hepatocellular carcinoma in Taiwan. Jpn J Cancer Res 2005, 96(12):1931-1936.

82. Weiskirchen R, Weiskirchen K, Chiono M, Snieder H, Kemp PM, Metcalfe JC, Carter ND, Spector TD: Genetic control of the circulating concentration of transforming growth factor type beta 1. Hum Mol Genet 1998, 7(1):93-97.

83. Granger DJ, Mosedale DE, Metcalfe JC: TGF-β in blood: a complex problem. Cytokine Growth Factor Rev 2000, 11(1-2):133-145.

84. Granger DJ, Mosedale DE, Metcalfe JC: TGF-β in blood: a complex problem. Cytokine Growth Factor Rev 2000, 11(1-2):133-145.

85. Wang H, Mengsteb S, Tag CG, Gao CF, Hellerbrand C, Lammert F, Grellner AM, Weiskirchen R: Transforming growth factor-beta gene polymorphisms are associated with progression of liver cirrhosis in patients with chronic hepatitis C infection. World J Gastroenterol 2005, 11(13):2929-1936.

86. Gewaltig J, Mangassser-Stephan K, Gartner C, Biesterfeld S, Grellner AM: Association of polymorphisms of the transforming growth factor-beta gene with the rate of progression of HCV-induced liver cirrhosis. Clinica Chimica Acta 2002, 316(1-2):83-94.

87. Tag CG, Mengsteb S, Hellerbrand C, Lammert F, Gresser AM, Weiskirchen R: Transforming growth factor-beta gene polymorphisms are associated with progression of liver cirrhosis in patients with chronic hepatitis C infection. Cytokine Growth Factor Rev 2005, 16(2):173-181.

88. Granger DJ, Mosedale DE, Metcalfe JC: TGF-β in blood: a complex problem. Cytokine Growth Factor Rev 2000, 11(1-2):133-145.

89. Granger DJ, Mosedale DE, Metcalfe JC: TGF-β in blood: a complex problem. Cytokine Growth Factor Rev 2000, 11(1-2):133-145.

90. Tag CG, Mengsteb S, Hellerbrand C, Lammert F, Gresser AM, Weiskirchen R: Transforming growth factor-beta gene polymorphisms are associated with progression of liver cirrhosis in patients with chronic hepatitis C infection. World J Gastroenterol 2005, 11(13):2929-1936.

91. Gewaltig J, Mangassser-Stephan K, Gartner C, Biesterfeld S, Grellner AM: Association of polymorphisms of the transforming growth factor-beta gene with the rate of progression of HCV-induced liver cirrhosis. Clinica Chimica Acta 2002, 316(1-2):83-94.

92. Tag CG, Mengsteb S, Hellerbrand C, Lammert F, Gresser AM, Weiskirchen R: Transforming growth factor-beta gene polymorphisms are associated with progression of liver cirrhosis in patients with chronic hepatitis C infection. World J Gastroenterol 2005, 11(13):2929-1936.

93. Weiskirchen R, Weiskirchen K, Chiono M, Snieder H, Kemp PM, Metcalfe JC, Carter ND, Spector TD: Genetic control of the circulating concentration of transforming growth factor type beta 1. Hum Mol Genet 1998, 7(1):93-97.

94. Granger DJ, Mosedale DE, Metcalfe JC: TGF-β in blood: a complex problem. Cytokine Growth Factor Rev 2000, 11(1-2):133-145.

95. Wang H, Mengsteb S, Tag CG, Gao CF, Hellerbrand C, Lammert F, Grellner AM, Weiskirchen R: Transforming growth factor-beta gene polymorphisms are associated with progression of liver cirrhosis in patients with chronic hepatitis C infection. World J Gastroenterol 2005, 11(13):2929-1936.

96. Gewaltig J, Mangassser-Stephan K, Gartner C, Biesterfeld S, Grellner AM: Association of polymorphisms of the transforming growth factor-beta gene with the rate of progression of HCV-induced liver cirrhosis. Clinica Chimica Acta 2002, 316(1-2):83-94.

97. Tag CG, Mengsteb S, Hellerbrand C, Lammert F, Gresser AM, Weiskirchen R: Transforming growth factor-beta gene polymorphisms are associated with progression of liver cirrhosis in patients with chronic hepatitis C infection. World J Gastroenterol 2005, 11(13):2929-1936.

98. Gewaltig J, Mangassser-Stephan K, Gartner C, Biesterfeld S, Grellner AM: Association of polymorphisms of the transforming growth factor-beta gene with the rate of progression of HCV-induced liver cirrhosis. Clinica Chimica Acta 2002, 316(1-2):83-94.

99. Tag CG, Mengsteb S, Hellerbrand C, Lammert F, Gresser AM, Weiskirchen R: Transforming growth factor-beta gene polymorphisms are associated with progression of liver cirrhosis in patients with chronic hepatitis C infection. World J Gastroenterol 2005, 11(13):2929-1936.

100. Gewaltig J, Mangassser-Stephan K, Gartner C, Biesterfeld S, Grellner AM: Association of polymorphisms of the transforming growth factor-beta gene with the rate of progression of HCV-induced liver cirrhosis. Clinica Chimica Acta 2002, 316(1-2):83-94.
in HIV-1 coreceptor accounts for resistance of some multi-
ply-exposed individuals to HIV-1 infection. Cell 1996, 
84(3):367-378.

109. Mullbauer M, Bosserhoff AK, Hartzmann A, Thaler WE, Weiss TS, 
Herfarth H, Lock G, Schömerich J, Hellerbrand C. A novel MCP-1 
gene polymorphism is associated with hepatic MCP-1 
expression and severity of HCV-related liver disease. Gastro-
enterology 2003, 125(4):1085-1093.

110. Erhardt A, Maschner-Olberg A, Mellenthin C, Kappert G, Adams O, 
Donner A, Willers R, Niederau C, Haussinger D; HFE mutations 
and chronic hepatitis C: H63D and C282Y heterozygosity 
are independent risk factors for liver fibrosis and cirrhosis. J 
Hepatol 2003, 38(3):375-382.

111. Geier A, Reugels M, Weiskirchen R, Wasmuth HE, Dietrich CG, 
Siewert E, Gartung C, Lorenzen J, Bosserhoff AK, Brugmann M; 
Common heterozygous hemochromatosis gene mutations 
are risk factors for inflammation and fibrosis in chronic hep-
atitis C. Hepatology 2003, 37(6):1546-1553.

112. Reynolds WF, Patel K, Planko S, Blatt LM, Nicholas JJ, McHutchison 
JG; A genotypic association implicates myeloperoxidase in 
the progression of hepatic fibrosis in chronic hepatitis C 
virus infection. Genes Immun 2002, 3(6):345-349.

113. Hennig BJ, Heffer J, Froodham AJ, Zhang L, Kleinerman P, Knopp S, 
Wright M, Thomas HC, Thursz M, Hill AV; Association of low-
density lipoprotein receptor polymorphisms and outcome of 
hepatitis C infection. Genes Immun 2002, 3:359-367.

114. Wozniak MA, Izhaki RF, Faragher EB, James MW, Ryder SD, Irving 
WL; Apolipoprotein E-e4 protects against severe liver dis-
ease caused by hepatitis C virus. Hepatology 2002, 
36(2):456-463.

115. Hsu LC, Park JMy, Zhang K, Luo JL, Kaufman RJ, Eckmann L, 
Guiney DG, Karin M; The protein kinase PKR is required for 
macrophage apoptosis after activation of Toll-like receptor 
4. Nature 2004, 428(6980):341-345.

116. Poltorak A, He X, Smirnova I, Zheng D, Hong T, Cheng G; Toll-
like receptors, endogenous ligands, and systemic 
autoimmune disease. Nature 2004, 428(6980):341-345.

117. Takekawa N, Ikejima K, Yamashina S, Hirose M, Shimizu H, Kitamura 
ET, Takei Y, Sato N, Thurman RG; Kupffer cell sensitization by 
endotoxin-tolerant mice have mutations in Toll-like 
receptor 4 (Tlr4). J Exp Med 1999, 189(6):615-625.

118. Rifkin IR, Leadbetter EA, Buzon E, Langlais G, Marshak-Rothstein A; 
Toll-like receptors, endogenous ligands, and systemic 
autoimmune disease. Immum Rev 2005, 204(1):27-42.

119. Takekawa N, Ikejima K, Yamashina S, Hirose M, Shimizu H, Kitamura 
ET, Takei Y, Sato N, Thurman RG; Kupffer cell sensitization by 
endotoxin-tolerant mice have mutations in Toll-like 
receptor 4 (Tlr4). J Exp Med 1999, 189(6):615-625.

120. Rifkin IR, Leadbetter EA, Buzon E, Langlais G, Marshak-Rothstein A; 
Toll-like receptors, endogenous ligands, and systemic 
autoimmune disease. Immum Rev 2005, 204(1):27-42.

121. Takekawa N, Ikejima K, Yamashina S, Hirose M, Shimizu H, Kitamura 
ET, Takei Y, Sato N, Thurman RG; Kupffer cell sensitization by 
endotoxin-tolerant mice have mutations in Toll-like 
receptor 4 (Tlr4). J Exp Med 1999, 189(6):615-625.

122. Bowie AG, Haga IR; The role of Toll-like receptors in the host 
response to viral and bacterial infections. Cell Res 
2005, 15(6):407-422.

123. Seno TLR1; Identification of 
CD14-deficient mice to endotoxin. J Immunol 
1999, 163(1):115-122.

124. Kawanishi T, Ikejima K, Yamashina S, Hirose M, Shimizu H, Kitamura 
ET, Takei Y, Sato N, Thurman RG; Kupffer cell sensitization by 
endotoxin-tolerant mice have mutations in Toll-like 
receptor 4 (Tlr4). J Exp Med 1999, 189(6):615-625.

125. Takekawa N, Ikejima K, Yamashina S, Hirose M, Shimizu H, Kitamura 
ET, Takei Y, Sato N, Thurman RG; Kupffer cell sensitization by 
endotoxin-tolerant mice have mutations in Toll-like 
receptor 4 (Tlr4). J Exp Med 1999, 189(6):615-625.

126. Bowie AG, Haga IR; The role of Toll-like receptors in the host 
response to viral and bacterial infections. Cell Res 
2005, 15(6):407-422.

127. Kawanishi T, Ikejima K, Yamashina S, Hirose M, Shimizu H, Kitamura 
ET, Takei Y, Sato N, Thurman RG; Kupffer cell sensitization by 
endotoxin-tolerant mice have mutations in Toll-like 
receptor 4 (Tlr4). J Exp Med 1999, 189(6):615-625.

128. Bowie AG, Haga IR; The role of Toll-like receptors in the host 
response to viral and bacterial infections. Cell Res 
2005, 15(6):407-422.

129. Sibille M, Hage T, Dufour A, Sibille M, Hage T, Dufour A; Toll-like 
receptors, endogenous ligands, and systemic 
autoimmune disease. Immum Rev 2005, 204(1):27-42.

130. Takekawa N, Ikejima K, Yamashina S, Hirose M, Shimizu H, Kitamura 
ET, Takei Y, Sato N, Thurman RG; Kupffer cell sensitization by 
endotoxin-tolerant mice have mutations in Toll-like 
receptor 4 (Tlr4). J Exp Med 1999, 189(6):615-625.

131. Takekawa N, Ikejima K, Yamashina S, Hirose M, Shimizu H, Kitamura 
ET, Takei Y, Sato N, Thurman RG; Kupffer cell sensitization by 
endotoxin-tolerant mice have mutations in Toll-like 
receptor 4 (Tlr4). J Exp Med 1999, 189(6):615-625.