Cell. Mol. Life Sci. 63 (2006) 3018–3029
1420-682X/06/243018-12
DOI 10.1007/s00018-006-6269-2

Cellular and Molecular Life Sciences

Review

Chitotriosidase: the yin and yang

L. Malaguarnera

Department of Biomedical Sciences, University of Catania, Via E. De Amicis 24, 95039 Trecastagni, Catania (Italy), Fax: +39 095 7807843, e-mail: lucmal@mbox.unict.it

Received 7 June 2006; received after revision 24 July 2006; accepted 21 September 2006
Online First 31 October 2006

Abstract. The enzyme chitotriosidase (ChT), the human analogue of chitinases from non-vertebrate species, is one of the most abundant and indicative proteins secreted by activated macrophages. Its enzymatic activity is elevated in serum of patients suffering from Gaucher's disease type 1 and in some other inherited lysosomal storage disorders, as well as in diseases in which macrophages are activated. The last decade has witnessed the appearance of a substantial number of studies attempting to unravel its cellular functions, which have yet not been fully defined. A great deal of progress has been made in the study of the physiological roles of ChT. This review is looks at the key areas of investigations addressed to further illuminate whether ChT activation might have different functional meanings in various diseases.

Keywords. Chitotriosidase, Gaucher's disease, β-thalassemia, *Plasmodium falciparum* malaria, immunity, atherosclerosis, neurodegenerative disorders, non-alcoholic steatohepatitis.

Introduction

Human chitotriosidase (ChT) is a member of the chitinase family and has the capability to hydrolyze chitin. Over the last decade, because of its clinical relavance, the enzyme ChT has received quite a lot of attention. In particular, it is a valuable diagnostic tool for monitoring the efficacy of therapy in Gaucher's disease (GD) or β-glucocerebrosidase deficiency [1]. More modest elevations in plasma ChT have been noted in some other inherited lysosomal storage disorders, especially sphingolipidoses such as Niemann Pick [2], GM1-gangliosidosis, and Krabbe's disease [3, 4]. In addition, it has been found that serum ChT activity is significantly increased in individuals suffering from atherosclerosis and is related to the severity of the atherosclerotic lesion, suggesting a possible role as an atherosclerotic extent marker [5–7]. Moreover, ChT is increased in patients suffering from malaria and in other hematological disorders where activated macrophages are involved [8, 9]. Novel findings indicate that ChT can also be regarded as an important player during the immunological response [10–12] and in other diseases in which the inflammatory processes play a relevant role [13–15]. Recombinant ChT has been found to inhibit hyphal growth of chitin-containing fungi, suggesting a physiological role in defense against chitin-containing pathogens [16, 17]. In this article, we enumerate the diseases with increased activity of ChT, although the list is not exhaustive, and seek to summarize what is known about ChT induction, and its physiological role in specific human diseases.

Structure of human ChT

ChT was the first discovered human analogue of chitinases. It belongs to the family 18 of glycosyl hydrolases [18, 19], and has the capability to hydrolyze chitin. Chitin is a glycopolymer of β-(1,4)-linked *N*-acetylglucosamine present as a structural component in the coating of many living species, such as the cell wall of fungi [20], the sheath of nematodes [21], and protozoan parasites [22],

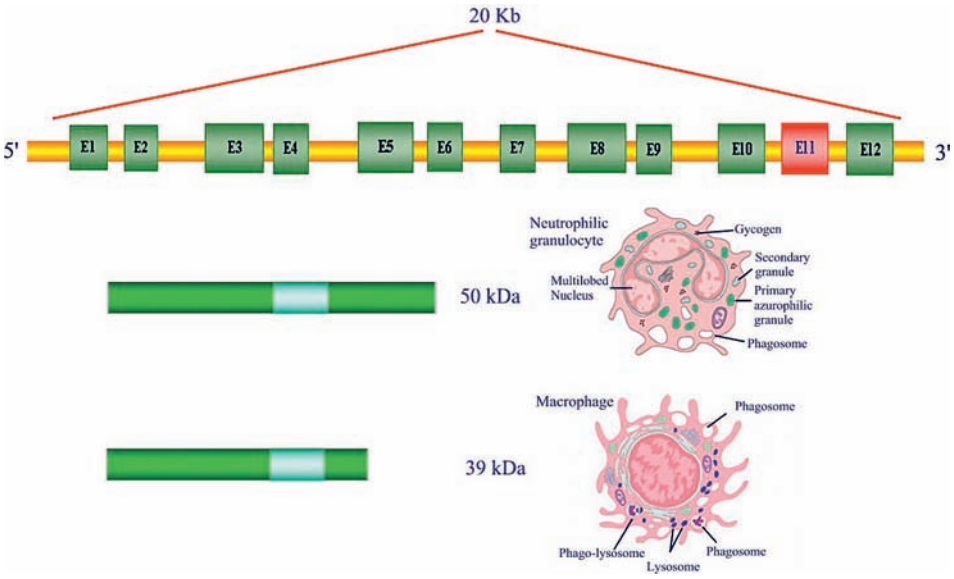

Figure 1. Genomic structure. ChT gene is composed of 12 exons and spans about 20 kb of genomic DNA. Exon 1 was defined as containing the first nucleotide of the longest chitotriosidase cDNA. The sizes of the exons range from 30 to 461 bp. The 71-bp exon 11 can be alternatively spliced. This exon is usually skipped in the splicing process, generating the predominant mRNA species encoding the 50-kDa protein stored in the granules of neutrophilic granulocyte progenitors. Exon 11 introduces a premature stop codon; the alternatively spliced mRNA encodes a 40-kDa ChT that is almost identical to the 39-kDa isoform generated by proteolytic processing of the 50-kDa ChT. The 39-kDa isoform is formed as the result of a lack of exon 11 skipping and accumulates in lysosomes of macrophages.

and in the gut lining of many insects [23]. Human ChT exhibits a remarkable sequence homology with other chitinases from viruses [22], plants, bacteria, fungi, nematodes and insects [18, 19]. The ChT gene is localized in chromosome 1q31-q32 [24]; ChT consists of 12 exons and spans approximately 20 kb of genomic DNA [19] (Fig. 1).

In human tissue ChT is heterogeneous with respect to its isoelectric point (p*I*) and molecular mass. The major isoform shows a molecular mass of 50 kDa and a heterogeneous p*I* of 5.0–7.2, this isoform contains the C-terminal chitin-binding domain and is synthesized by neutrophilic granulocyte progenitors and stored in their granules [19, 25] (Fig. 1). The cloning of its cDNA from a macrophage library showed that the 50-kDa form can be converted to a C-terminally truncated 39-kDa isoform post-translationally or by RNA processing [16, 26]; this truncated isoform has a pI of 8.1 [26] and accumulates in the lysosomes [27] (Fig. 1). It has recently been noted that the C-terminal domain of 50-kDa ChT mediates a strong binding of this enzyme to chitin, enabling it not only to cleave chitotriose but also hydrolyzes colloidal chitin to yield chitobiose, a feature that is not shown by the 39-kDa isoform [27]. The N termini of both isoforms are identical as disclosed by the cloned ChT cDNA.

The crystal structures of human ChT described by Fusetti and coworkers [28] have provided further clues for understanding the possible functions of the protein. The structure reveals that a long cleft runs across one face of the protein and that this cleft is lined with solvent-exposed aromatic residues [28]. Binding experiments in which a complex of ChT with the chito-oligosaccharide *N*-acetylglucosamine-2 (NAG2) was created, followed by modeling of a longer chito-oligosaccharide, revealed that the active sites were able to accommodate longer chitin polymers, which agrees with its ability to degrade various forms of polymeric chitin [28]. The ability to bind long chitin polymers and the relatively open active site architecture implies that this enzyme functions as an endochitinase rather than an exochitinase [28].

Other human members of the chitinase protein family have been identified: oviductin (human oviduct-specific glycoprotein) [29], human cartilage glycoprotein 39 (HCgp-39/YKL40) [30], YKL39 [31], and TSA 1902 [32]. Although significant homology was observed between the four human chitinases, glycosyl hydrolase activity has only been identified in ChT. These human chitinases lack the catalytic acidic amino acid residues. The biological function of this family has not been fully elucidated. It has been suggested that they might have a role in a tissue-remodeling processes [30], or chemotaxis [33, 34].

Another mammalian chitinase, named acidic mammalian chitinases (AMCase) [17], is characterized by an acidic p*I* and extreme stability at acid pH [35]. AMCase is relatively abundant in the gastrointestinal tract and lung, supporting a possible role as a food processor in stomach and its involvement in lung inflammation [17, 35]. This protein has 52% sequence identity with the human macrophage chitinase and also contains the additional α/β folds

[17]. Given the different expression patterns and the fact that this additional mammalian chitinase has a pH optimum of around 2, it is likely that it plays a different role compared with its human analogue ChT.

ChT and GD

GD is an autosomal recessive inherited lysosomal disorder. It is characterized biochemically by a deficiency of the lysosomal enzyme β-glucosidase (glucocerebrosidase), which is responsible for the cleavage of β-glycoside bonds resulting in glucose and ceramide residues [36].
Deficiency of this enzyme leads to the accumulation of glucocerebrosides in the lysosomes of some cells, referred as Gaucher's cells [36, 37].
The disease can be classified into three clinical types. Type 1, the most common, is the chronic non-neuropathic form of the disease, which shows highly variable signs and symptoms and a variable course, with visceral and skeletal involvement. Accumulation of lipid-laden macrophages (Gaucher's cells) in the affected individuals causes the development of hepatosplenomegaly, bone lesions and, occasionally, neurological abnormalities [36].
Biochemical abnormalities secondary to bone or visceral involvement are common in patients with GD. Thus, regular assessment of biochemical variables is valuable in monitoring both the course of the disease and the response to treatment. The most prominent biochemical abnormality is the elevation of plasma ChT [1]. Hollak and coworkers in the 1994 first reported that ChT activity was strikingly elevated in plasma samples of untreated type 1 GD patients. The increased plasma level of this enzyme was studied in relation with other markers such as β3-hexosaminidase, angiotensin converting enzyme, and lysozyme [1]. These investigators found a slight elevation in ChT activity even in asymptomatic glucocerebrosidase-deficient individuals. Enzyme-replacement therapy administrating purified placental glucocerebrosidase has proven to be effective in improving the clinical and hematological parameters of GD patients. In a subsequent study Hollak and coworkers [38] proposed that a reduction in ChT activity of less than 15% after 12 months of enzyme-replacement therapy, in combination with an insufficient response of at least one clinical parameter, should suggest a dose increase. Therefore, ChT is a useful marker for monitoring GD patients for its correlation with disease burden and its sensitivity to treatment. The rapid decline in ChT levels after the start of enzyme supplementation therapy, preceding objective clinical improvement, indicates that the production of ChT is not a simple function of the number of lipid-laden macrophages, but may reflect a particular state of activation of macrophages leading to an excessive production of ChT. Thus, the decrease in ChT activity during administration of glucocerebrosidase may cause an alteration in this state rather than a decrease in 'Gaucher's cell' mass. Interestingly, ChT is a marker that does not overlap between patients and controls [38]. A drawback in the use of ChT as a marker of GD, however, is that 3–5% of individuals in the general population have no activity due to the presence of a null allele in the ChT gene [16].

ChT polymorphism

A recessive inherited deficiency in ChT activity is frequently encountered in different ethnic groups. The defect consists of 24-bp duplication in exon 10 that activates a cryptic 39 splice site in the same exon, generating an abnormally spliced mRNA with an in-frame deletion of 87 nucleotides (Fig. 2). The spliced mRNA encodes

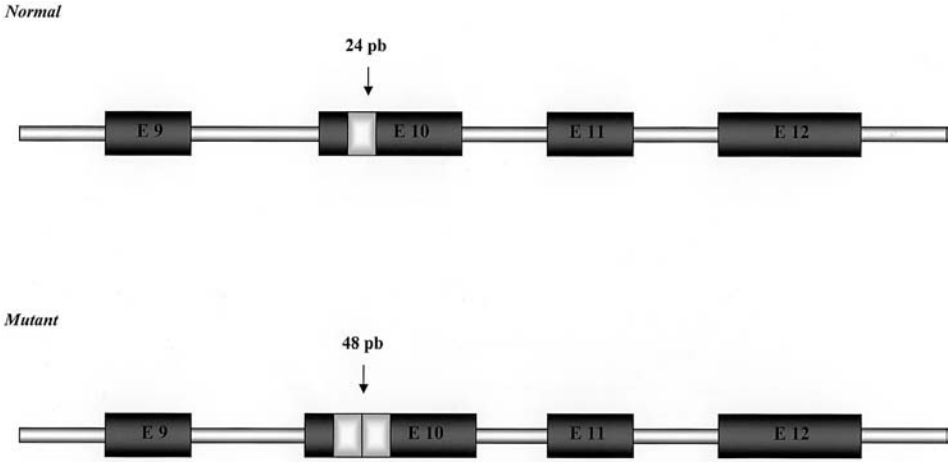

Figure 2. Defect in ChT mRNA in ChT-deficient individuals. Upper panel: Normal ChT gene; lower panel: difference in the mutant ChT gene. The mutant gene contains 24-bp duplication in exon 10. The arrows indicate the 24-bp fragment in the normal gene and the 48-bp fragment in the mutant gene.

Table 1. Frequencies of wild-type and mutant alleles in different ethnic groups.

Population	Genotype			Reference
	Wild type	Heterozygote mutant	Homozygote mutant	
Dutch	58.5%	35.1%	6.4%	[16]
Ashkenazi Jewish	60.3%	33.8%	5.9%	[16]
Portuguese	60.0%	37.3%	2.7%	[39]
Spanish	54.3%	39.6%	6.03%	[7]
Sicilian	51.01%	44.54%	5.45%	[42]
Sardinian	65.56%	32.71%	3.73%	[42]
Benin	100%	0%	0%	[42]
Burkina Faso	98%	2%	0%	[42]

an enzymatically inactive protein that lacks an internal stretch of 29 amino acids [16] (Fig. 2). This ChT mutant allele has been found in 33–35% of Ashkenazi Jewish and Dutch individuals, respectively, whereas both populations were about 6% homozygous for this allele [16]. Additional studies performed in a Portuguese population confirmed that approximately 6% and 40% of the general population are homozygous and heterozygous, respectively, for this ChT activity deficiency (Table 1) [39]. The multi-ethnic occurrence and prevalence of ChT gene mutation suggested that the enzyme is redundant in man, and that this mutation is relatively ancient in the evolution. In the subjects having the mutant allele, the activity of ChT is undetectable in all materials tested including plasma, urine, leukocytes, and tissues [4, 16], indicating that this mutation is the predominant cause of ChT deficiency [16]. According to the relationship between genotype and phenotype, ChT deficiency in humans appears as an autosomal incompletely dominant disorder, with no activity in homozygous subjects for the defective allele and approximately half-normal activities in heterozygous subjects. Cultured macrophages of ChT-deficient individuals contain very little mRNA and secrete almost no ChT protein.

Studies of the ChT structure have contributed to clarifying why deletion of this region inactivates the enzyme. In the inactive enzyme residues Val344–Gln372 are missing; these residues correspond to the C-terminal half of helix α7, the entire strand β8, and almost the entire β8-α8 loop [28]. The internal deletion preventing the formation of a proper TIM-barrel conformation produces a mutant protein showing no chitinolitic activity [16]. Interestingly, Trp358, which lies at the end of strand β8, is completely conserved in all active family 18 chitinases [28]. Inspection of chitinase structures in complex with chito-oligosaccharides [40, 41] shows that this tryptophan serves as an 'anvil' onto which the sugar at the -1 position is pressed, whereas specific hydrogen bonds with other residues may force the sugar into the boat conformation required for the attack of the *N*-acetyl group on the anomeric carbon [40]. Thus, deletion of Trp358 could in itself further lead a completely inactive enzyme [28].

Could susceptibility to parasitic disease be dependent on the ChT allele composition?

A high incidence of ChT gene mutation was also found in other study carried out in some ethnic groups from the Mediterranean area [42]. In these findings, a heterozygote frequency for the duplication of 24 base pairs in exon 10 of 44–54% in Sicily and 32.71% in Sardinia was found, whereas corresponding values for homozygous ChT deficiency were 5.45% and 3.73%, respectively. In contrast, in African subjects from Benin and in Burkina Faso, a low incidence of ChT mutation was found (heterozygous 0% and 2%, respectively) and no subject was homozygous for ChT deficiency [42].

The low incidence of heterozygotes for ChT-mutated allele in African subjects suggested the hypothesis that the persistence of parasitic diseases could have favored the maintenance of the wild-type ChT gene in sub-Saharan regions. In fact, the subjects bearing the mutant allele exhibit an elevated susceptibility to infections sustained by parasites. This observation was in accordance with the findings reported by Choi et al. [43], who showed that in a total of 216 individuals from South India genotyped for ChT, the homozygous condition for the defective allele (the HH variant ChT 1 genotype) was associated with the absence of plasma ChT activity and with an elevated susceptibility to human *Wuchereria bancrofti* filarial infection, confirming the importance of ChT in the protection against the chitin-containing pathogens [43]. Therefore, the susceptibility to parasitic disease may be dependent on the ChT allele composition.

The hypothesis that the persistence of parasitic diseases favored the maintenance of the wild-type ChT is based also on the finding showing that in a group of 40 children with acute *Plasmodium falciparum* malaria, the ChT activity was in the range of the African population, whereas in another group of 27 children these levels were significantly higher [8, 42]. These observations indicate that in Africa the wild-type ChT gene maintains a relevant function in the monocyte-macrophage response. However, the higher levels of plasma ChT activity of African subjects might do not only reflect the homozygous wild-type allele

condition, but suggests that among the African population, several subjects may be affected by silent parasite infections. Why the mutation in the ChT gene occurs with such high incidence in different ethnic groups is an intriguing question, and further studies are necessary to determine whether some selective pressure may have influenced ChT polymorphism in the different ethnic groups.

ChT synthesis in human diseases

ChT and malaria

Increased levels of ChT were also observed in patients with malaria [8]. A clinical feature in patients infected by *Plasmodium falciparum* malaria is the occurrence of anemia, which has been related to red cell destruction [44], phagocytosis and hypersplenism [45]. Particularly, red cell destruction triggers ChT overproduction due to the accumulation of iron and erythrocyte membrane degradation products within the macrophages. Plasma levels of ChT were found related with the hematological parameters, such as thrombocytopenia degree and serum ferritin levels [8, 46], suggesting that the increase of plasma ChT in malaria reflects an activation of the reticulo-endothelial system. The mechanisms leading to the activation of ChT in malaria patients seem to be the same as those described earlier in GD patients.

ChT and immunity

A large body of evidence strongly supports the idea that the ChT gene could have more pleiotropic effects in innate immunity than previously appreciated. Macrophages are generally considered to be important elements in natural resistance in most, if not all, organs and are strategically placed to protect the microenvironment in which they are situated. In this context, a great deal of attention should be focused on the fact that ChT is produced by the macrophages themselves. ChT is not a house-keeping enzyme [19], and macrophages are able to produce large amounts of this enzyme under specific circumstances. On some occasions it is the main protein secreted, representing approximately 1% of the total protein secretion [19].

The involvement of ChT in the immunological response emerged from the study demonstrating that IFN-γ, TNF-α and LPS up-regulated ChT gene expression in human macrophages, whereas IL-10 led to remarkable suppression of ChT expression [12]. Additionally, prolactin, which is structurally related to several cytokines and, importantly, modulates the expression of genes crucial for leukocytes function [47–49], was shown to up-regulate ChT gene expression in human macrophages [10].

Recent studies have revealed that human macrophages cultured with GM-CSF expressed increased ChT transcripts [50]. Administration of GM-CSF produces beneficial effects in patients with fungal infections [51], promoting the release of ChT from PMNs via exocytosis of specific granules [50]. Further evidence confirming that ChT can be regarded as a mediator during the immunological response comes from the finding that, in chimpanzees, IL-12 injection is associated with enhanced ChT activity [52]. Since IL-12 is a potent immunoregulatory cytokine that is crucially involved in a wide range of infectious diseases [53], it is reasonable that the elevated levels of IL-12 modulate the macrophage activity, stimulating their microbicidal function through a pathway involving ChT activity [8]. This idea can be supported by the studies showing that, in malaria, protective immunity is mediated by a cascade of events involving IL-12 [54].

ChT has been shown to play a role also in defense against chitinous human pathogens [17]. In fact, the high incidence of human filariasis is associated with an increased deficiency in ChT [43]. ChT inhibits growth of *Cryptococcus neoformans*, causes hyphal tip lysis in *Mucor rouxii*, and prevents the occurrence of hyphal switch in *Candida albicans* [17], through its ability to degrade both colloidal chitin and chitin in the cell wall of the fungal pathogen. Moreover, it has been found that ChT activity rises in plasma of neonates with systemic candidiasis [55]. Parasitic nematodes synthesize chitin during several stages of their lifecycle, and the human ChT seems to interfere with these processes. In neutropenic mouse models of systemic candidiasis and systemic aspergillosis, recombinant human ChT was shown to improve survival [56]. Overall, these findings suggest a possible use of recombinant chitotriosidase to treat life-threatening fungal infections [56]. Finally, adding to the list of findings demonstrating a role of ChT in the immune response, functionally important variants in ChT alleles have been shown to influence the susceptibility to infections with Gram-negative bacteria [57].

Other members of chitinase family are also likely to be involved in the immune response, *e.g.* oviductin, which may play a role in the protection of the tubal epithelium [58]; HCgp-39, which drives immune regulatory responses in humans limiting the catabolic effects of inflammatory cytokines [59, 60]; and YKL39, which may be involved in the pathological process of osteoarthritis and rheumatoid arthritis [61]. In addition, the recent demonstration that AMCase is involved in the pathology of an aeroallergen asthma model [62] and the data on the defense functions of ChT against chitin-containing pathogens underscore the important role of the family of chitinase proteins in the human immune system, and explain the emerging interest in them as targets for therapeutic interventions. Nevertheless, the inhibition of chitinase activity as a therapeutic approach should be dealt with carefully as there is a risk that important anti-pathogenic activities of chitinases are eliminated as a consequence of total chitinases inhibition.

ChT and bronchial asthma

The finding that AMCase play an important role in the pathogenesis of bronchial asthma [62] and that several polymorphisms and haplotypes of AMCase showed a strong association with pediatric asthma [63] prompted investigation of whether ChT was involved in the pathogenesis of bronchial asthma. The amino acid variants Gly102Ser and Ala442Gly as well as the 24-bp duplication within ChT showed no association with bronchial asthma in Caucasian children [64]. This result suggested that the two human chitinases have different functions in human diseases even though they have the same substrate. Interestingly, AMCase is induced via a T helper-2 (Th2)-specific, IL-13-mediated pathway in macrophages and epithelial cells [62], whereas, so far, no involvement of ChT in Th2 immunity has been described. Additionally, the expression pattern of both chitinases varies completely; ChT is exclusively produced by phagocytes, whereas AMCase is expressed in alveolar macrophages and in the gastrointestinal tract [65]. The different role in human asthma of ChT and AMCase may be, at least in part, dependent on these latter findings.

ChT and thalassemia

ChT was found to be increased in patients with β-thalassemia, a hematological disorder characterized by the genetic defect of β-globin chains synthesis, resulting in unproductive erythropoiesis and an enormous expansion of the reticulo-endothelial system.

High plasma levels of ChT, comparable to the levels found in GD patients, were found in 10% of patients with β-thalassemia major in whom, following an intense transfusion regimen, serum ferritin levels and urinary iron excretion were increased. Modest elevation of ChT was found in the patients with β-thalassemia intermedia, who did not receive transfusion and had normal or only slightly increased serum ferritin level [9, 66]. Variable increases in ChT activity have been also observed in studies performed in Israeli patients with β-thalassemia major [66]. The continuous blood transfusions lead to a remarkable iron accumulation, so that thalassemia major is regarded as a disease of iron overload [67]. Since it is noteworthy that iron accumulation causes lysosomal instability due to peroxidative injury [68], the increase of plasma ChT activity in β-thalassemia major patients could be related to iron-mediated damage to the lysosomal apparatus.

Although entirely different in their molecular basis, GD and β-thalassemia share biochemical abnormalities that indicate an important role of activated macrophages into the physiopathology of both these disorders. In GD, lack of β-glucocerebrosidase renders phagocytes unsuitable for the turnover of the membrane lipids of the phagocytosed blood-formed elements. As a consequence, macrophages become lipid-laden and are transformed into 'Gaucher's cells'. In addition, intracellular accumulation of ferritin and iron is commonly seen in 'Gaucher's cells', and plasma ferritin levels are increased in GD patients [36]. In β-thalassemia the amount of abnormal and/or transfused red blood cells exceeds the degrading capacity of macrophages. Phagocytes of β-thalassemia patients are consequently laden with membranous lipid material. Therefore, similarly to that seen in GD, the increased ChT production in β-thalassemia might reflect macrophage activation related to intracellular iron overload, storage of erythrocyte membrane breakdown products and oxidation of excess α-hemoglobin subunits [9]. Both conditions are also characterized by an excess of free oxygen radicals production and increased consumption of vitamin E, which reflects the existence of activated macrophages [69]. High levels of serum macrophage CSF, which controls and enhances several monocyte-macrophage functions, has been shown in β-thalassemia [70] and GD [9, 71]. In view of the similarities between substrate-laden phagocytes in GD and β-thalassemia patients, it is not surprising that both pathological cells share a common biomarker like ChT.

ChT and atherosclerosis

Recently, Boot et al. [6] reported that ChT activity is elevated up to 55-fold in extracts of atherosclerotic tissue, showing a clear connection between ChT expression and lipid-laden macrophages inside human atherosclerotic vessel wall, as in GD. Serum ChT activity was shown to be related to the severity of the atherosclerotic lesions, suggesting a possible role as a marker of atherosclerotic extension. High serum ChT activity in patients with atherosclerosis demonstrates the presence of activated macrophages in these subjects. In other studies, patients with atherothrombotic stroke (ATS) and ischemic heart disease (IHD) were reported to have significantly higher ChT activities than the control group [6]. However, ATS subjects had higher ChT activity than IHD subjects, suggesting that in the subjects with ATS the atherosclerosis process is wider than in the subjects with IHD, whose atherosclerosis is localized more specifically in the coronary vessels. This was confirmed by the observation that, in the ATS group, ChT activity was related to carotid stenosis, in accordance with a clinical picture featured by a more widespread atherosclerosis [6]. Interestingly, no detectable serum ChT activity was found in the subjects homozygous for the defective allele. ChT activity was significantly higher in homozygous subjects for the major allele than in heterozygous subjects for the defective allele [7]. In addition, the increase in serum ChT activity was found to be age dependent. This phenomenon could

be explained by the ongoing accumulation of lipid-laden macrophages during the gradual progression of atherosclerosis in relation to age. A similar relationship with age for ChT activity was previously described in subjects with different lysosomal disorders and in the general population [4, 5].

A large body of clinical and experimental evidence indicates that inflammation plays a central role in the beginning of the atherosclerosis process and in the mechanism underlying the development and progression of the atherosclerosis complications, plaque rupture and subsequent thrombosis [72–74]. Macrophages are present in all phases of atherogenesis, and are markers of atherosclerotic plaques formation [75]. Proceeding inflammation results in an increase in numbers of macrophages and lymphocytes, both markers of the ongoing inflammation. Macrophage accumulation localized in the supraaortic and coronary vessels is associated with increased serum ChT activity, which reflects the state of activation of macrophages within atherosclerotic lesions, suggesting that ChT augmentation could influence the synthesis of crucial components of the extracellular matrix in the vessel wall [5].

It should be emphasized that the average ChT activity in serum remained constant after 6 months of lipid-lowering treatment with either atorvastatin or bezafibrate, suggesting that LDL-cholesterol and triglyceride reduction obtained with both drugs did not modify the macrophage ChT expression in these subjects [7]. Therefore, plasma lipid correction does not seem to interfere in the ChT expression level *in vivo*, supporting the idea that ChT activity cannot be used as a biological marker of atherosclerotic plaque modification related to hypolipidemic treatment [7].

ChT and neurodegenerative disorders

Recent investigations suggest that ChT could play a crucial role also in pathological conditions such as cerebrovascular dementias in which the inflammatory process is activated.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder resulting in the loss of higher cognitive function. Senile plaque is a key pathological feature of AD and it is composed of insoluble β-amyloid (Aβ) fibril deposits, astrocytes, and degenerating neurons. A feature in the brain of AD patients is the presence of activated microglia and astroglia, releasing pro-inflammatory cytokines and chemokines [76]. In addition, the increased production of reactive oxygen (ROS) and nitrogen species, such as superoxide anion (O_2^-) and nitric oxide (NO) generate peroxynitrite, leading to a condition commonly referred to as 'oxidative stress' [77, 78]. Therefore, the presence of inflammatory mediators highly expressed in the vicinity of Aβ deposits in the brain of AD patients, strongly indicate the contribution of inflammation in the pathogenesis of AD [79]. As well as AD, the pathophysiological events involved in ischemic cerebrovascular dementia (CvD) in the development of brain ischemia and multi-infarct cognitive impairment appear due to secondary mechanisms substantially mediated by inflammation [80]. A large body of evidence implicates impaired energy metabolism and oxidative damage in the pathogenesis of both AD and CvD [76]. The oxidative damage observed in CvDs is identical to that seen in lipid-associated disorders. In fact, in the pathogenesis of AD the oxidative damage to lipids preceded Aβ deposition in a transgenic mouse model of AD [81]. Similarly, in CvD, cessation of blood flow in cerebral stroke triggers an oxidative stress resulting in a massive production of ROS, which induces increased levels of lipid peroxidation. Therefore, the result of the augmentation of ChT levels also in AD and CvD is not surprising.

The analysis of the levels of the expression of several cytokines (IL-16, IL-18, TGF-β1) revealed that: (i) IL-16 was markedly elevated in CvD compared with AD patients and was significantly and positively correlated with ChT, suggesting that IL-16 could influence ChT expression. The evidence that IL-16 plays a chemoattractant and immunomodulatory role in chronic inflammatory disorders in the brain [82], could explain why chemoattracted macrophages activate their gene to produce ChT. (ii) The production of IL-18 resulted increased, and positively correlated with ChT, in both AD and CvD patients, suggesting that the high levels of pro-inflammatory mediators including ChT and O_2^- enhance cytokine release from brain resident cells, aggravating the inflammatory response.(iii) The expression of TGF-β1 in AD patients was inversely correlated with the expressions of ChT, suggesting that the action of TGF-β1 counteracts the effects of pro-inflammatory mediators, further confirming that ChT reflects the severity of inflammation [15]. Interestingly, an increased activity of ChT has been shown in patients with ischemic stroke [83], which directly correlates with stroke severity [84]. Conversely, other investigators have observed that, in most advanced clinical stages of AD, the levels of ChT activity did not present any statistically significant differences with respect to the controls [85]. These contradictory data may be related to the different stage of the disease. One interesting thought for the future could be to find out if ChT is only a marker or whether it contributes to the progression of cerebrovascular disorders.

ChT and non-alcoholic steatohepatitis

Recently, evidence has been presented that ChT increases significantly in non-alcoholic steatohepatitis (NASH)

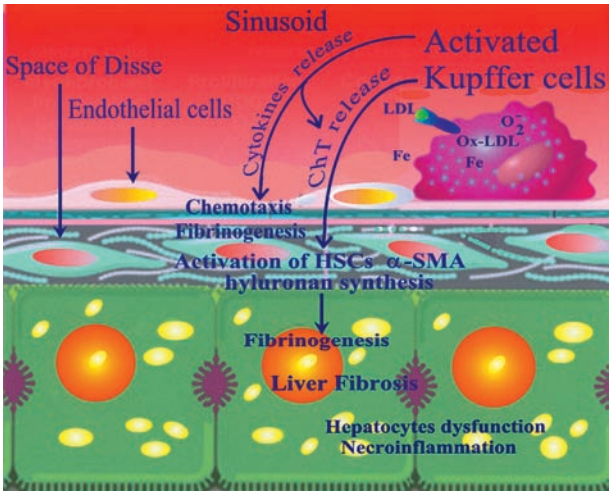

Figure 3. Mechanism by which ChT could be involved in the induction of fibrogenesis. ChT released by activated Kupffer cells could activate HSCs to synthesize collagen whose overproduction leads to hepatic fibrosis and cirrhosis.

and Kupffer cells are the only hepatic source responsible for ChT mRNA expression [13, 14].

NASH is a clinico-pathological condition characterized by a necroinflammatory disorder with fatty infiltration of hepatocytes. This disorder can occur in association with the use of numerous drugs, parenteral nutrition, gastric bypass surgery, and inherited metabolic disorders including obesity, dyslipidemias, type 2 diabetes and other forms of the insulin-resistance syndrome [86, 87]. A crucial event in the initiation of NASH involves lipid accumulation and lipid peroxidation in the hepatocytes, followed by Kupffer cells and hepatic stellate cells (HSCs) activation. Since Kupffer cells possess scavenger receptors they can be activated by exposure to products of lipid peroxidation [88, 89]. The increased levels of ChT expression in Kupffer cells were related with O_2^- production and lipid peroxides levels. An added factor in the progression of NASH could involve the release of cytokines by hepatocytes and Kupffer cells. In response to liver cell injury/inflammation, activated Kupffer cells are a source of proinflammatory cytokines, which could induce ChT production. Therefore, a contribution of ChT release to the activation by non-parenchymal cells cannot be excluded, particularly as the biological effects of ChT are regulated by the release of cytokines [11, 12]. However, ROS formation, lipid peroxidation and cytokines production do not appear to be sufficient for affecting changes in ChT levels. In fact, the highest levels of ChT were observed in patients with the highest degree of hepatic iron accumulation [13]. It is noteworthy that iron is a potent catalyst for lipid peroxidation and, thus, participates in hyperlipidemia. It appears that the increase of ChT in Kupffer cells might reflect an iron-mediated damage of lysosomes, suggesting that the physiological function of ChT probably contributes in regulating the acute-phase response as an adaptive metabolic response to iron.

ChT levels were correlated with α-SMA expression, strongly suggesting that ChT could be involved in the modulation of the extracellular matrix in hepatic tissue, affecting cell adhesion and chemotaxis during the tissue remodeling processes that take place during fibrogenesis (Fig. 3) [14]. These data are consistent with *in vitro* studies showing that Kupffer cell medium stimulates collagen production and proliferation by isolated HSCs and elicits an activated morphology [90]. This activation is associated with increased synthesis and secretion of several extracellular matrix components, including type I collagen [91], laminin [92], fibronectin [93] and proteoglycans [94]. In most types of liver disease encountered in clinical practice, HSC proliferation/activation and subsequent fibrogenesis is considered to constitute part of the expected healing response to hepatocyte necrosis and inflammation. ChT released by activated Kupffer cells could activate HSCs to synthesize collagen, the overproduction of which leads to hepatic fibrosis and cirrhosis. The parallel increases of ChT levels in Kupffer cells and α-SMA in HCSs strongly suggest that this enzyme can be regarded as a mediator of HSC activation in the liver of NASH patients. Furthermore, exposure to pro-oxidants or end products of lipid peroxidation activates the transcription of collagen genes [95]. Recently, a vertebrate chitin synthase has been identified, which is supposed to create short chitin stretches that are essential to initiate hyaluronan synthesis [96, 97]. The glucosaminoglycan hyaluronan level is higher in the liver of NASH patients, and seems associated with liver fibrosis [98]. It has been hypothesized that ChT recognizes hyaluronan (precursor) as a substrate and interferes with its synthesis, which could affect local hyaluronan concentrations and consequently influence the extent of cell migration in the injured hepatic tissue. These data further reinforce the idea that ChT can also be regarded as an important player during inflammation [12]. Nevertheless, additional studies should be directed to determine whether ChT can be proposed as mediator of fibrogenesis capable of directly activating quiescent cells or whether it requires the presence of other as yet unidentified 'activating' stimuli before exerting its effects. It is con-

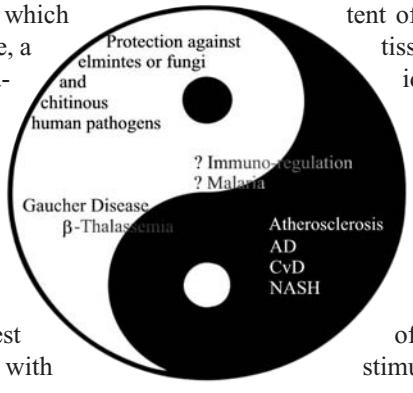

Figure 4. Yin–Yang balance: implications of ChT synthesis in different contexts.

ceivable that the activity of this enzyme also enhances in other liver diseases, in which stimulation of Kupffer cells by portal vein endotoxin may cause the release of cytokines and chemokines, hepatocyte hyper-metabolism, and activation of HSCs [99].

Open questions

Although we do not yet fully understand the implications of ChT synthesis in response to chitinous pathogens, the recent concept of its function as 'more than just antifungicidal' seems justified. Synthesis of ChT probably occurs in most innate immune responses against fungi, bacteria and other non-viral pathogens. In the context of infectious diseases, it is likely that ChT activity can be both harmful and advantageous for the host organism. In addition, it cannot be excluded that ChT augmentation could also have detrimental consequences in those condition in which it is regarded as a biochemical marker of macrophage activation (Fig. 4). In support of this view are the recent investigations confirming that ChT can be regarded as a mediator of the inflammatory response and could be involved in the progression of fibrosis in several degenerative disorders (Fig. 4). The general concept of the multiplicity of ChT involvement in human diseases discussed in this review may stimulate the development of new ideas and experiments leading to a deeper understanding, not only on the biochemical mechanisms inducing ChT regulation, but also on the consequences of the increases in ChT levels and on the impact of the homozygous wild-type allele condition in different contexts. In conclusion, the factors that tip this Yin–Yang balance require further study possibly using selective and specific chitinase inhibitors.

1 Hollak, C. E., van Weely, S., van Oers, M. H. and Aerts, J. M. (1994) Marked elevation of plasma chitotriosidase activity. A novel hallmark of Gaucher disease. J. Clin. Invest. 93, 1288–1292.

2 Brinkman, J., Wijburg, F. A., Hollak, C. E., Groener, J. E., Verhoek, M., Scheij, S., Aten, J., Boot, R. G. and Aerts, J. M. (2005) Plasma chitotriosidase and CCL18: early biochemical surrogate markers in type B Niemann-Pick disease. J. Inherit. Metab. Dis. 28, 13–20.

3 Michelakakis, H., Dimitriou, E. and Labadaridis, I. (2004) The expanding spectrum of disorders with elevated plasma chitotriosidase activity: an update. J. Inherit. Metab. Dis. 27, 705–706.

4 Guo, Y., He, W., Boer, A. M., Wevers, R. A., de Bruijn, A. M., Groener, J. E., Hollak, C. E., Aerts, J. M., Galjaard, H. and van Diggelen, O. P. (1995) Elevated plasma chitotriosidase activity in various lysosomal storage disorders. J. Inherit. Metab. Dis. 18, 717–722.

5 Boot, R. G., van Achterberg, T. A., van Aken, B. E., Renkema, G. H., Jacobs, M. J., Aerts, J. M. and de Vries, C. J. (1999) Strong induction of members of the chitinase family of proteins in atherosclerosis: chitotriosidase and human cartilage gp-39 expressed in lesion macrophages. Arterioscler. Thromb. Vasc. Biol. 19, 687–694.

6 Artieda, M., Cenarro, A., Ganan, A., Jerico, I., Gonzalvo, C., Casado, J. M., Vitoria, I., Puzo, J., Pocovi, M. and Civeira, F. (2003) Serum chitotriosidase activity is increased in subjects with atherosclerosis disease. Arterioscler. Thromb. Vasc. Biol. 23, 1645–1652.

7 Canudas, J., Cenarro, A., Civeira, F., Garci-Otin, A. L., Aristegui, R., Diaz, C., Masramon, X., Sol, J. M., Hernandez, G. and Pocovi, M. (2001) Chitotriosidase genotype and serum activity in subjects with combined hyperlipidemia: effect of the lipid-lowering agents, atorvastatin and bezafibrate. Metabolism 50, 447–450.

8 Barone, R., Simporè J., Malaguarnera, L., Pignatelli, S. and Musumeci, S. (2003) Plasma chitotriosidase activity in acute *Plasmodium falciparum* malaria. Clin. Chim. Acta 331, 79–85.

9 Barone, R., Di Gregorio, F., Romeo, M. A., Schiliro, G. and Pavone, L. (1999) Plasma chitotriosidase activity in patients with β-thalassemia. Blood Cells Mol. Dis. 15, 1–8.

10 Malaguarnera, L., Musumeci, M., Licata, F., Di Rosa, M., Messina, A. and Musumeci, S. (2004) Prolactin induces chitotriosidase gene expression in human monocyte-derived macrophages. Immunol. Lett. 94, 57–63.

11 Malaguarnera, L., Musumeci, M., Di Rosa, M., Scuto, A. and Musumeci, S. (2005) Interferon-gamma, tumor necrosis factor-alpha, and lipopolysaccharide promote chitotriosidase gene expression in human macrophages. J. Clin. Lab. Anal. 19, 128–132.

12 Di Rosa, M., Musumeci, M., Scuto, A., Musumeci, S. and Malaguarnera, L. (2005) Effect of interferon-gamma, interleukin 10, lipopolysaccharide and tumor necrosis factor-alpha on chitotriosidase synthesis in human macrophages. Clin. Chem. Lab. Med. 43, 499–502.

13 Malaguarnera, L., Di Rosa, M., Zambito, A. M., Dell'Ombra, N., Nicoletti, F. and Malaguarnera, M. (2006) Chitotriosidase gene expression in Kupffer cells of non-alcoholic fatty liver disease patients. Gut 55, 1313–1320.

14 Malaguarnera, L., Di Rosa, M., Zambito, A. M., Dell'Ombra, N., Di Marco, R. and Malaguarnera, M. (2006) Potential role of chitotriosidase gene in nonalcoholic fatty liver disease evolution. Am. J. Gastroenterol. 101, 2060–2069.

15 Di Rosa, M., Dell'Ombra, N., Zambito, A. M., Malaguarnera, M., Nicoletti, F. and Malaguarnera, L. (2006) Chitotriosidase and inflammatory mediator levels in Alzheimer's disease and cerebrovascular dementia. Eur. J. Neurosci. 23, 2648–2656.

16 Boot, R. G., Renkema, G. H., Verhoek, M., Strijland, A., Bliek, J., de Meulemeester, T. M., Mannens, M. M. and Aerts, J. M. (1998) The human chitotriosidase gene. Nature of inherited enzyme deficiency. J. Biol. Chem. 273, 25680–25685.

17 Boot, R. G., Blommaart, E. F., Swart, E., Ghauharali-van der Vlugt, K., Bijl, N., Moe, C., Place, A. and Aerts, J. M. (2001) Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. J. Biol. Chem. 276, 6770–6778.

18 Henrissat, B. and Bairoch, A. (1993) New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem. J. 293, 781–788.

19 Boot, R. G., Renkema, G. H., Strijland, A., van Zonneveld, A. J. and Aerts, J. M. (1995) Cloning of a cDNA encoding chitotriosidase, a human chitinase produced by macrophages. J. Biol. Chem. 270, 26252–26256.

20 Kuranda, M. J. and Robbins, P. W. (1991) Chitinase is required for cell separation during growth of *Saccharomyces cerevisiae*. J. Biol. Chem. 266, 19758–19767.

21 Wu, Y., Egerton, G., Underwood, A. P., Sakuda, S. and Bianco, A. E. (2001) Expression and secretion of a larval-specific chitinase (family 18 glycosylhydrolase) by the infective stages of the parasitic nematode, *Onchocerca volvulus*. J. Biol. Chem. 276, 42557–42564.

22 Vinetz, J. M., Dave, S. K., Specht, C. A., Brameld, K. A., Xu, B., Hayward, R. and Fidock, D. A. (1999) The chitinase

PfCHT1 from the human malaria parasite *Plasmodium falciparum* lacks proenzyme and chitin-binding domains and displays unique substrate preferences. Proc. Natl. Acad. Sci. USA 96, 14061–14066.
23 Cohen, E. (1993) Chitin synthesis and degradation as targets for pesticide action. Arch. Insect Biochem. Physiol. 22, 245–261.
24 Eiberg, H. and Den Tandt, W. R. (1997) Assignment of human plasma methylumbelliferyl-tetra-*N*-acetylchitotetraoside hydrolase or chitinase to chromosome 1q by a linkage study. Hum. Genet. 101, 205–207.
25 Boussac, M. and Garin, J. (2000) Calcium-dependent secretion in human neutrophils: a proteomic approach. Electrophoresis 21, 665–672.
26 Renkema, G. H., Boot, R. G., Muijsers, A. O., Donker-Koopman, W. E. and Aerts, J. M. (1995) Purification and characterization of human chitotriosidase, a novel member of the chitinase family of proteins. J. Biol. Chem. 270, 2198–21202.
27 Renkema, G. H., Boot, R. G., Strijland, A., Donker-Koopman, W. E., van den Berg, M., Muijsers, A. O. and Aerts, J. M. (1997) Synthesis, sorting, and processing into distinct isoforms of human macrophage chitotriosidase. Eur. J. Biochem. 244, 279–285.
28 Fusetti, F., von Moeller, H., Houston, D., Rozeboom, H. J., Dijkstra, B. W., Boot, R. G., Aerts, J. M. and van Aalten, D. M. (2002) Structure of human chitotriosidase. Implications for specific inhibitor design and function of mammalian chitinase-like lectins. J. Biol. Chem. 277, 25537–25544.
29 Arias, E. B., Verhage, H. G. and Jaffe, R. C. (1994) Complementary deoxyribonucleic acid cloning and molecular characterization of an estrogen-dependent human oviductal glycoprotein. Biol. Reprod. 51, 685–694.
30 Hakala, B. E., White, C. and Recklies, A. D. (1993) Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. J. Biol. Chem. 268, 25803–25810.
31 Hu, B., Trinh, K., Figueira, W. F. and Price, P. A. (1996) Isolation and sequence of a novel human chondrocyte protein related to mammalian members of the chitinase protein family, J. Biol. Chem. 271, 19415–19420.
32 Saito, A., Ozaki, K., Fujiwara, T., Nakamura, Y. and Tanigami, A. (1999) Isolation and mapping of a human lung-specific gene, TSA1902, encoding a novel chitinase family member. Gene 239, 325–331.
33 Owhashi, M., Arita, H. and Hayai, N. (2000) Identification of a novel eosinophil chemotactic cytokine (ECF-L) as a chitinase family protein. J. Biol. Chem. 275, 1279–1286.
34 Malinda, K. M., Ponce, L., Kleinman, H. K., Shackelton, L. M. and Millis, A. J. (1999) Gp38k, a protein synthesized by vascular smooth muscle cells, stimulates directional migration of human umbilical vein endothelial cells. Exp. Cell Res. 250, 168–173.
35 Chou, Y. T, Yao, S., Czerwinski, R., Fleming, M., Krykbaev, R., Xuan, D., Zhou, H., Brooks, J., Fitz, L., Strand, J., Presman, E., Lin, L., Aulabaugh, A. and Huang, X. (2006) Kinetic characterization of recombinant human acidic mammalian chitinase. Biochemistry. 45, 4444–4454.
36 Beutler, E. and Grabowski, D. A. (2001) Gaucher disease. in: The Metabolic and Molecular Basis of Inherited Disease (Scriver, C. R., Beaudet, A. L., Sly, W. S., Valle, D., Eds), pp. 3635–3656. McGraw-Hill, New York.
37 Esplin, J. A. (1994) Overview: Gaucher disease, molecular, medicine and therapeutics. Gauch. Clin. Perspect. 2, 1–16.
38 Hollak, C. E., Maas, M. and Aerts, J. M. (2001) Clinically relevant therapeutic endpoints in type 1 Gaucher disease. J. Inherit. Metab. Dis. 24 [Suppl. 2], 97–105.
39 Rodrigues, M. R., Sa Miranda, M. C. and Amaral, O. (2004) Allelic frequency determination of the 24-bp chitotriosidase duplication in the Portuguese population by real-time PCR. Blood Cells Mol. Dis. 33, 362–364.
40 van Aalten, D. M., Komander, D., Synstad, B., Gaseidnes, S., Peter, M. G. and Eijsink, V. G. (2001) Structural insights into the catalytic mechanism of a family 18 exochitinase. Proc. Natl. Acad. Sci. USA 98, 8979–8984.
41 Papanikolau, Y., Prag, G., Tavlas, G., Vorgias, C. E., Oppenheim, A. B. and Petratos, K. (2001) High resolution structural analyses of mutant chitinase A complexes with substrates provide new insight into the mechanism of catalysis. Biochemistry 40, 11338–11343.
42 Malaguarnera, L., Simpore, J., Prodi, D. A., Angius, A., Sassu, A., Persico, I., Barone, R. and Musumeci, S. (2003) A 24-bp duplication in exon 10 of human chitotriosidase gene from the sub-Saharan to the Mediterranean area: role of parasitic diseases and environmental conditions. Genes Immun. 4, 570–574.
43 Choi, E. H., Zimmerman, P. A., Foster, C. B., Zhu, S., Kumaraswami, V., Nutman, T. B. and Chanock, S. J. (2001) Genetic polymorphisms in molecules of innate immunity and susceptibility to infection with Wuchereria bancrofti in South India. Genes Immun. 2, 248–253.
44 Weatherall, D. J. and Abdalla, S. (1982) The anaemia of *Plasmodium Falciparum* malaria. Br. Med. Bull. 38, 147–151.
45 Weiss, L. (1990) The spleen in malaria: the role of barrier cells. Immunol. Lett. 25, 165–172.
46 Kwiatkowski, D. (1999) The molecular genetic approach to malarial pathogenesis and immunity. Parassitologia 41, 233–240.
47 Bazan, J. F. (1989) A novel family of growth factor receptors: a common binding domain in the growth hormone, prolactin, erythropoietin and IL-6 receptors, and the p75 IL-2 receptor beta-chain. Biochem. Biophys. Res. Commun. 164, 788–795.
48 O'Neal, K. D., Schwarz, L. A. and Yu-Lee L-Y. (1992) Prolactin receptor gene expression in lymphoid cells. Mol. Cell. Endocrinol. 82, 127–135.
49 Kooijman, R., Hooghe-Peters, E. L. and Hooghe, R. (1996) Prolactin, growth hormone, and insulin-like growth factor-1 in the immune system. Adv. Immunol. 63, 377–454.
50 Hashimoto, S., Suzuki, T., Dong, H. Y., Yamazaki, N. and Matsushima, K. (1999) Serial analysis of gene expression in human monocytes and macrophages. Blood 94, 837–844.
51 Jones, T. C. (1999) Use of granulocyte-macrophage colony stimulating factor (GM-CSF) in prevention and treatment of fungal infections. Eur. J. Cancer 35 Suppl. 3, S8–10.
52 Lauw, F. N., te Velde, A. A., Dekkers, P. E., Speelman, P., Aerts, J. M., Hack, C. E., van Deventer, S. J. and van der Poll, T. (1999) Activation of mononuclear cells by interleukin-12: an *in vivo* study in chimpanzees. J. Clin. Immunol. 19, 231–232.
53 Romani, L., Puccetti, P. and Bistoni, F. (1997) Interleukin-12 in infectious diseases. Clin. Microbiol. Rev. 10, 611–636.
54 Malaguarnera, L., Imbesi, R. M., Pignatelli, S., Simpore, J., Malaguarnera, M. and Musumeci, S. (2002) Increased levels of interleukin-12 in *Plasmodium falciparum* malaria: correlation with the severity of disease. Parasite Immunol. 24, 387–389.
55 Labadaridis, I., Dimitriou, E., Theodorakis, M., Kafalidis, G., Velegraki, A. and Michelakakis, H. (2005) Chitotriosidase in neonates with fungal and bacterial infections. Arch. Dis. Child. Fetal Neonatal Ed. 90, F531–532.
56 van Eijk, M., van Roomen, C. Pl, Renkema, G. H., Bussink, A. P., Andrews, L., Blommaart, E. F., Sugar, A., Verhoeven, A. J., Boot, R. G. and Aerts, J. M. (2005) Characterization of human phagocyte-derived chitotriosidase, a component of innate immunity. Int. Immunol. 17, 1505–1512.
57 Lehrnbecher, T., Bernig, T., Hanisch, M., Koehl, U., Behl, M., Reinhardt, D., Creutzig, U., Klingebiel, T., Chanock, S. J. and Schwabe, D. (2005) Common genetic variants in the interleukin-6 and chitotriosidase genes are associated with the risk for serious infection in children undergoing therapy for acute myeloid leukemia. Leukemia 19, 1745–1750.
58 Briton-Jones, C., Lok, I. H., Yuen, P. M., Chiu, T. T., Cheung, L. P. and Haines, C. (2001) Regulation of human oviductin mRNA expression *in vivo*. Fertil. Steril. 75, 942–946.

59 van Bilsen, J. H., van Dongen, H., Lard, L. R, van der Voort, E. I, Elferink, D. G, Bakker, A. M., Miltenburg, A. M., Huizinga, T. W., de Vries, R. R. and Toes, R. E. (2004) Functional regulatory immune responses against human cartilage glycoprotein-39 in health vs. proinflammatory responses in rheumatoid arthritis. Proc. Natl. Acad. Sci. USA 101, 17180–17185.
60 Ling, H. and Recklies, A. D. (2004) The chitinase 3-like protein human cartilage glycoprotein 39 inhibits cellular responses to the inflammatory cytokines interleukin-1 and tumour necrosis factor-alpha. Biochem. J. 380, 651–659.
61 Tsuruha, J., Masuko-Hongo, K., Kato, T., Sakata, M., Nakamura, H., Sekine, T., Takigawa, M. and Nishioka, K. (2002) Autoimmunity against YKL-39, a human cartilage derived protein, in patients with osteoarthritis. J. Rheumatol. 29, 1459–1466.
62 Zhu, Z., Zheng, T., Homer, R. J., Kim, Y. K., Chen, N. Y., Cohn, L., Hamid, Q. and Elias, J. A. (2004) Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. Science 304, 1678–1682.
63 Bierbaum, S., Nickel, R., Koch, A., Lau, S., Deichmann, K. A, Wahn, U., Superti-Furga, A. and Heinzmann, A. (2005) Polymorphisms and haplotypes of acid mammalian chitinase are associated with bronchial asthma. Am. J. Respir. Crit. Care Med. 172, 1505–1509.
64 Bierbaum, S., Superti-Furga, A. and Heinzmann, A. (2006) Genetic polymorphisms of chitotriosidase in Caucasian children with bronchial asthma. Int. J. Immunogenet. 33, 201–204.
65 Boot, R. G., Bussink, A. P., Verhoek, M., de Boer, P. A., Moorman, A. F. and Aerts, J. M. (2005) Marked differences in tissue-specific expression of chitinases in mouse and man. J. Histochem. Cytochem. 53, 1283–1292.
66 Altarescu, G., Rudensky, B., Abrahamov, A., Goldfarb, A., Rund, D., Zimran, A. and Elstein, D. (2002) Plasma chitotriosidase activity in patients with beta-thalassemia. Am. J. Hematol. 71, 7–10.
67 Giardina, P. J. and Grady, R. W. (1995) Chelation therapy in β-thalassemia: the benefits and limitations of desferrioxamine. Semin. Hematol. 32, 304–312.
68 Tong Mak, I. and Weglicki, E. B. (1985) Characterization of iron-mediated peroxidative injury in isolated hepatic lysosomes. J. Clin. Invest. 75, 58–63.
69 Rachmilewitz, E. A., Kornberg, A. and Acker, M. (1982) Vitamin E deficiency due to increased consumption in β-thalassemia and in Gaucher disease. Ann. N. Y. Acad. Sci. 393, 336–347.
70 Wiener, E., Wanachiwanawin, W., Chinprasertsuk, S., Siripanyaphinyo, U., Mawas, F., Fucharoen, S. and Wickramasinghe, S. N. (1996) Increased serum levels of macrophage colony-stimulating factor (M-CSF) in alpha- and beta-thalassaemia syndromes: correlation with anaemia and monocyte activation. Eur. J. Haematol. 57, 364–369.
71 Aerts, J. M. and Hollak, C. E. (1997) Plasma and metabolic abnormalities in Gaucher's disease. Baillieres Clin. Haematol. 10, 691–709.
72 Ross, R. (1999) Atherosclerosis: an inflammatory disease. N. Engl. J. Med. 340, 115–126.
73 Young, J. L., Libby, P. and Schonbeck, U. (2002) Cytokines in the pathogenesis of atherosclerosis. Thromb. Haemost. 88, 554–567.
74 Libby, P., Ridker, P. M. and Maseri, A. (2002) Inflammation and atherosclerosis. Circulation 105, 1135–1143.
75 Moreno, P. R., Falk, E., Palacios, I. F., Newell, J. B., Fuster, V. and Fallon, J. T. (1994) Macrophage infiltration in acute coronary syndromes: implications for plaque rupture. Circulation 90, 775–778.
76 Chong, Z. Z., Li, F. and Maiese, K. (2005) Stress in the brain: novel cellular mechanisms of injury linked to Alzheimer's disease. Brain Res. Brain Res. Rev. 49, 1–21.
77 Luth, H. J., Munch, G. and Arendt, T. (2002) Aberrant expression of NOS isoforms in Alzheimer's disease is structurally related to nitrotyrosine formation. Brain Res. 953, 135–143.
78 Wong, A., Luth, H. J., Deuther-Conrad, W., Dukic-Stefanovic, S., Gasic-Milenkovic, J., Arendt, T. and Munch, G. (2001) Advanced glycation endproducts colocalize with inducible nitric oxide synthase in Alzheimer's disease. Brain Res. 920, 32–40.
79 McGeer, E. G. and McGeer, P. L. (2001) Innate immunity in Alzheimer's disease: a model for local inflammatory reactions. Mol. Interv. 1, 22–29.
80 Dirnagl, U., Iadecola, C. and Moskowitz, M. A. (1999) Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci. 22, 391–397.
81 Pratico, D., Uryu, K., Leight, S., Trojanoswki, J. Q. and Lee, V. M. (2001) Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. J. Neurosci. 21, 4183–4187.
82 Schwab, J. M., Nguyen, T. D., Meyermann, R. and Schluesener, H. J. (2001) Human focal cerebral infarctions induce differential lesional interleukin-16 (IL-16) expression confined to infiltrating granulocytes, $CD8^+$ T-lymphocytes and activated microglia/macrophages. J. Neuroimmunol. 114, 232–241.
83 Palasik, W., Fiszer, U., Lechowicz, W., Czartoryska, B., Krzesiewicz, M. and Lugowska, A. (2005) Assessment of relations between clinical outcome of ischemic stroke and activity of inflammatory processes in the acute phase based on examination of selected parameters. Eur Neurol. 53, 188–193.
84 Sotgiu, S., Barone, R., Zanda, B., Arru, G., Fois, M. L., Arru, A., Rosati, G., Marchetti, B. and Musumeci, S. (2005) Chitotriosidase in patients with acute ischemic stroke. Eur. Neurol. 54, 149–153.
85 Casal, J. A., Robles, A. and Tutor, J. C. (2003) Serum markers of monocyte/macrophage activation in patients with Alzheimer's disease and other types of dementia. Clin. Biochem. 36, 553–556.
86 Chitturi, S. and Farrell, G. C. (2001) Etiopathogenesis of nonalcoholic steatohepatitis. Semin. Liver Dis. 21, 27–41.
87 Chitturi, S., Abeygunasekera, S., Farrell, G. C., Holmes-Walker, J., Hui, J. M., Fung, C., Karim, R., Lin, R., Samarasinghe, D., Liddle, C., Weltman, M. and George, J. (2002) NASH and insulin resistance: insulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology 35, 373–379.
88 Van Berkel, T. J. C., de Rijke, Y. B. and Kruijt, J. K. (1991) Different fat *in vivo* of oxidatively modified-LDL and acetylated-LDL in rats. Recognition by various scavenger receptors on Kupffer cells and endothelial liver cells. J. Biol. Chem. 266, 2282–2289.
89 De Rijke, Y. B. and Van Berkel, T. J. (1994) Rat liver Kupffer and endothelial cells express different binding proteins for modified low-density lipoproteins. Kupffer cells express a 95-kDa-membrane protein as a specific binding site for oxidized low-density lipoproteins. J Biol Chem. 269, 824–827.
90 Matsuoka, M., Zhang, M. Y. and Tsukamoto, H. (1990) Sensitisation of hepatic lipocytes by high fat diet to stimulatory effects of Kupffer cell-derived factors: Implications for fibrogenesis. Hepatology 11, 173–182.
91 Friedman, S. L. (1990) Cellular sources of collagen and regulation of collagen production in liver. Semin. Liver Dis. 10, 20–29.
92 Maher, J. J, Friedman, S. L, Roll, F. J. and Bissell, D. M. (1988) Immuno-localization of laminin in normal rat liver and biosynthesis of laminin by hepatic lipocytes in primary culture. Gastroenterology 94, 1053–1062.
93 Rieder, H., Ramadori, G., Dienes, H. P. and Meyer zum Buschenfelde, K. H. (1987) Sinusoidal endothelial cells from guinea pig liver synthesize and secrete cellular fibronectin *in vitro*. Hepatology 7, 856–864.
94 Schafer, S., Zerbe, O. and Gressner, A. M. (1987) The synthesis of proteoglycans in fat-storing cells of rat liver. Hepatology 7, 680–687.

95 Bedossa, P., Houglum, K., Trautwein, C., Holstege, A. and Chojkier, M. (1994) Stimulation of collagen a1(I) gene expression is associated with lipid per-oxidation in hepatocellular injury: A link to tissue fibrosis? Hepatology 19, 1262–1271.
96 Meyer, M. F. and Kreil, G. (1996) Cells expressing the DG42 gene from early *Xenopus* embryos synthesize hyaluronan. Proc. Natl. Acad. Sci. USA 93, 4543–4547.
97 Varki, A. (1996) Does DG42 synthesize hyaluronan or chitin? A controversy about oligosaccharides in vertebrate development. Proc. Natl. Acad. Sci. USA 93, 4523–4525.
98 Sakugawa, H., Nakayoshi, T., Kobashigawa, K., Yamashiro, T., Maeshiro, T., Miyagi, S., Shiroma, J., Toyama, A., Nakayoshi, T., Kinjo, F. and Saito, A. (2005) Clinical usefulness of biochemical markers of liver fibrosis in patients with non-alcoholic fatty liver disease. World, J. Gastroenterol. 11, 255–259.
99 Lieber, C. S. (2004) Alcoholic fatty liver: Its pathogenesis and mechanism of progression to inflammation and fibrosis. Alcohol 34, 9–19.