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MINIREVIEW

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## Antibodies Against Oxidized LDL – Theory and Clinical Use

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Received April 10, 2000

Accepted September 4, 2000

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### Summary

Modification of low density lipoprotein (LDL) particles due to oxidation, glycation and binding of advanced glycation end-products (AGEs) or malondialdehyde (MDA, a final product of lipid peroxidation) is considered most important in the process of atherogenesis. Oxidatively modified LDL are distinguished by another receptor type, which was discovered on the surface of macrophages and was called the scavenger receptor. Uncontrolled intake of LDL converts macrophages to foam cells; their accumulation under the vascular endothelium is considered as the first stage of atherosclerosis. Oxidation of LDL is a complex process taking place in both the extra- and intracellular space. At the end of this oxidative process, modified LDL particles show chemotactic, cytotoxic and immunogenic properties. Oxidized LDL express a large number of epitopes and cause production of polyclonal autoantibodies against these products, especially against apoB<sub>100</sub> modified by MDA and 4-hydroxynonenal. IgoxLDL (antibodies against oxidized LDL) can be demonstrated either directly in intimal lesions or as a component of circulating immune complexes. IgoxLDL do not form a homogeneous group but a varied mixture of antibodies-isoantibodies caused by HDL and LDL polymorphism, antibodies against the lipid phase of LDL and antibodies against modified apoB<sub>100</sub> of the immunoglobulin class IgA or IgG. Antibodies against oxLDL were found in many diseases other than atherosclerosis such as diabetes mellitus, renovascular syndrome, uremia, rheumatic fever, morbus Bechtjerev or lupus erythematoses. Newborns have practically the same levels of IgoxLDL as their mothers; however, these values did not differ from those in the healthy population of non-pregnant women of the same age. The decrease in IgoxLDL titer was very slow and lasted many months; that is why this parameter cannot be considered suitable for describing the rapid changes during oxidative stress of the organism. Positive correlation of IgoxLDL with antiphospholipids and other antibodies was repeatedly demonstrated; their determination can thus be used as a marker for the description of total production of autoantibodies in various diseases. The changes and correlations of IgoxLDL, anti-β-2-glycoprotein I IgG and antiphospholipid antibodies support the immunological link between thrombotic and atherosclerotic processes in the human body.

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### Key words

IgoxLDL • Low density lipoprotein • Autoantibodies • Atherosclerosis • Oxidative stress

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## Introduction

Atherosclerosis is the most frequent cause of death in the civilized world. That is why its possible cause, epidemiology and pathogenesis, as well as the possibilities of its early diagnosis and treatment have been intensively studied for many years. A lot of time has passed since Anitschow fed rabbits with a high-cholesterol diet, thus causing atherosclerotic changes in their arteries; cholesterol was identified as one of the most important risk factors of atherosclerosis development (Anitschow and Chalатов 1913, Anitschow 1915). Opinions about the significance of cholesterol in atherogenesis were then gradually specified: it was found that the principal role is played by the distribution and transport of cholesterol – the lipoprotein and apoprotein type. Atherosclerosis is now considered as a complex process, which – according to the results of recent studies – already has its origin in the first years of life (Stary 1987).

Cholesterol itself is neither toxic nor antigenic. The same cannot be said about LDL particles transporting cholesterol: if they are altered, they become harmful for the organism. It is this modification due to oxidation, glycation and binding of AGEs (advanced glycation end-products) or MDA (malondialdehyde, a final product of lipid peroxidation) which is considered most important in the process of atherogenesis. The interaction of modified LDL with scavenger receptors on the surface of macrophages leads to the formation of foam cells, which represent the first phase of the atherosclerotic process (Parthasarathy and Rankuin 1992, Witzum 1994, Ross 1996, Berliner and Heinecke 1997).

Oxidative modification of LDL causes their antigenicity. Antibodies against oxidized LDL (IgoxLDL) are a heterogeneous group of autoantibodies, which are directed against oxidatively modified LDL particles. The introduction of their determination into clinical practice extended the possibilities to investigate immunological aspects of atherosclerosis development. These auto-antibodies are not exclusively specific for atherosclerosis, but their rise parallels the increased formation of oxidatively modified LDL particles. The aim of this review is to acquaint the reader with the history of IgoxLDL research, the theoretical basis of their formation and the use of their determination in clinical practice.

### Brief history of IgoxLDL

IgoxLDL were discovered by chance by Beumont in a patient with multiple myeloma and hyperlipidemia (Beumont 1965, Beumont and

Lorenzelli 1967). In the same year, similar antibodies were described by a team of physicians from the USA in a rapidly progressing case of familiar hyperlipidemia with xanthomatosis which did not respond to therapy (Lewis and Page 1965).

On the basis of the methodological possibilities available at that time, the authors first isolated an immune complex and called it the x-factor; this complex was then found in fractions of  $\beta$ -lipoproteins and  $\gamma$ -globulins. This finding was explained by Beumont as autoimmune hyperlipidemia which is manifested by an increased occurrence of immune complexes in the blood and causes a decrease in lipoprotein clearance. This observation and its explanation were not generally accepted.

Ten years later, a scientific team from Switzerland extended Beumont's observations by finding anti-LDL autoantibodies in other diseases, which were not caused by atherosclerosis. Autoantibodies against oxidized LDL were detected in patients with primary chronic polyarthritis and breast cancer (Riesen and Nosedá 1975). According to Salonen *et al.* (1992), there is a positive correlation between the IgoxLDL titer and atherosclerosis development. Other studies have documented a relationship between atherosclerosis and immunological processes.

### Physiology and biochemistry of LDL

During IgoxLDL formation, the definitive role is played by low-density lipoproteins (LDL) belonging to a group of lipoproteins which are secreted by the liver. It supplies body cells with endogenous cholesterol, which is – after binding of LDL to specific membrane receptors – transported into the cells and subsequently metabolized (Esterbauer 1995). On the basis of ultrastructural studies, it was found that LDL are spherical particles 22-26 nm in diameter, density 1.019-1.063 g/ml and relative molecular weight of 2.5 million daltons: LDL is composed of 22.3 % phospholipids, 5.9 % triglycerides, 9.6 % free cholesterol, 42.2 % cholesteryl esters and 22 % proteins, mainly apolipoprotein B<sub>100</sub> (apoB<sub>100</sub>). The total number of fatty acids, which are bound in different lipids of one LDL particle, is about 2600. Approximately one half of them are represented by polyene (polyunsaturated) fatty acids, especially linolenic, arachidonic and docosahexaenoic acids. Both cholesterol and polyene fatty acids are well known to be very susceptible to peroxidation by free radicals. LDL are protected against oxidative modification by a series of antioxidants. One of the most important antioxidants is the lipid-soluble  $\alpha$ -tocopherol (vitamin E). Each LDL particle contains approximately seven molecules of this substance. Other important lipid-soluble antioxidants are carotenoids,

retinoids and ubiquinol (coenzyme Q<sub>10</sub>). In comparison with  $\alpha$ -tocopherol, these antioxidants are present in LDL particles in significantly lower amounts.

Free cholesterol and phospholipids are the basis of the amphoteric properties of LDL-particles – they contain both lipo- and hydrophilic groups. Lysine residues of apoB<sub>100</sub> cause their high susceptibility to aldehyde binding and oxidative modification (Szondy *et al.* 1978). The size of LDL particles rather varies and is determined genetically (Roheim and Asztalos 1995). According to their size, we distinguish two phenotypes. Persons with phenotype A (75 %) have LDL with a diameter between 26 and 27 nm and lower density. People with phenotype B (25 %) have smaller LDL particles (19–22 nm in diameter) and their density is higher. From the epidemiological point of view, they represent a group with a higher risk of coronary sclerosis and myocardial infarction (Roheim and Asztalos 1995). These particles are also more susceptible to oxidation.

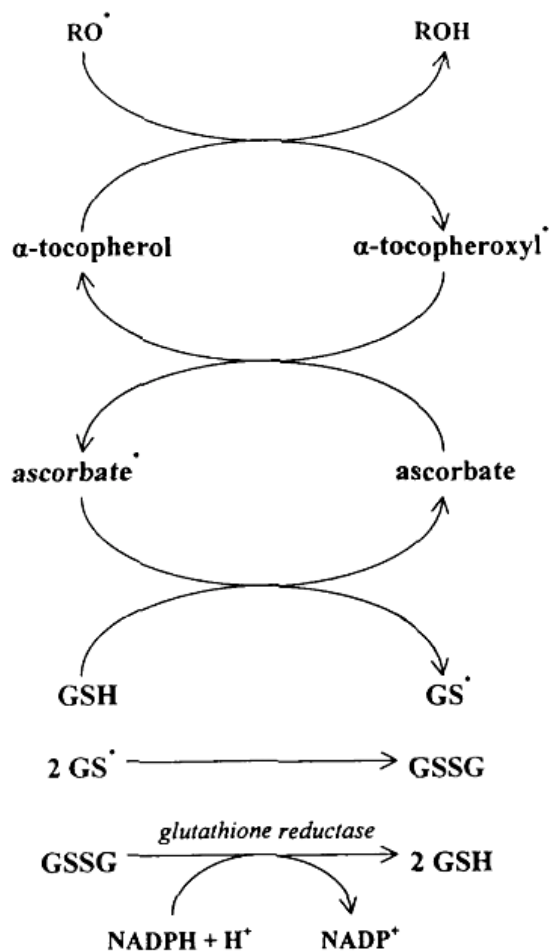
The size of LDL can be influenced – besides hereditary factors – by diet, obesity, diabetes mellitus, hypertension and by adverse effects of the environment such as stress or insufficient exercise. On the other hand, the diameter of LDL can be diminished by some drugs ( $\beta$ -blockers or clofibrate). The mechanism of increased cardiovascular risk has been discussed; it can be linked, for example, to a lower clearance of small LDL particles or a decreased number of LDL receptors. If LDL are retained in the circulation, they are exposed to free radicals; due to a relatively high content of antioxidants in the blood plasma, LDL are usually oxidized after their penetration across the endothelium, i.e. in the arterial wall. Here, small LDL also penetrate more easily and are then oxidized in the vascular wall. Patients with a high lipid content in the blood show a decreased amount of small LDL in the plasma after a low-fat diet. That is why a certain reversibility of LDL size can be expected.

A comparison of human and animal LDL (or their apolipoproteins) revealed that human and swine LDL have very similar physical chemical properties. LDL of monkeys have a larger surface, but the immunological similarity is much higher between humans and monkeys (cross-reactivity 80–85 %) than between humans and pigs (cross-reactivity only 35 %).

#### Pathophysiology of LDL oxidation

Oxidation of LDL is a complex process taking place in both the extra- and intracellular space. There are still many details of this process which are to be clarified. *In vivo* lipid peroxidation was observed especially in

tissue macrophages, endothelial cells and smooth muscle cells (Griffith *et al.* 1988, Lugheed *et al.* 1991, Yla-Herttuala 1991). Hemoglobin, hypochlorous acid, ceruloplasmin, lipoxygenase or peroxidase appeared to be effective oxidants.



**Fig. 1.** Regeneration of vitamin E; GSH – reduced glutathione, GSSG – oxidized glutathione, GS – thiyl radical, NADPH, NADP<sup>+</sup> – nicotine amide adenine dinucleotide phosphate (reduced and oxidized forms)

Lipid peroxidation can be observed *in vitro* as a change of the lag phase of LDL oxidation stimulated by Cu<sup>2+</sup> ions. This measurement reflects the ability of LDL to take care of oxidative stress (Esterbauer *et al.* 1996a, Gieseg and Esterbauer 1994). Oxidized LDL can be determined by immunochemical methods, electrophoresis or spectrophotometrically (Esterbauer and Jürgens 1993, Gieseg and Esterbauer 1994, Zawadzki *et al.* 1989, Wieland *et al.* 1992, Zima *et al.* 1998a).

Oxidatively modified LDL can be distinguished by another receptor type, which was discovered on the surface of macrophages and has been called the scavenger receptor. Uncontrolled intake of LDL converts macrophages to foam cells; their accumulation under the vascular endothelium is considered to be the first stage of atherosclerosis. Products of lipid peroxidation in an atherosclerotic plaque act chemotactically on blood monocytes and cause the activation of cyclooxygenase.

Oxidation of LDL proceeds in three phases (Esterbauer *et al.* 1990, Esterbauer and Jürgens 1993, Esterbauer and Ramos 1996).

#### Lag phase

During the lag phase which lasts about 2-3 hours, the amount of antioxidants in these particles decreases. This can be explained by their consumption in reactions with free radicals; the defense against oxidation of polyunsaturated fatty acids decreases. The most important antioxidant in LDL particles is  $\alpha$ -tocopherol (vitamin E) which needs ascorbic acid (vitamin C), glutathione and NADPH in the hydrophilic compartment for its regeneration (Fig. 1). Exogenous antioxidants are equally effective as those of endogenous origin if their absorption in the gastrointestinal tract is sufficient. Oxygen and free radicals cause the rapid consumption of antioxidants, which form the first defensive line against free radicals.

#### Progression phase

The progression phase lasts 1-2 hours during which rapid and total lipid peroxidation occurs. Free radicals start a peroxidation cascade when polyunsaturated acids are gradually changed to conjugated dienes, hydroperoxides and other products leading finally to their fragmentation and formation of alkanes and reactive aldehyde compounds (Fig. 2).

#### Decomposition phase

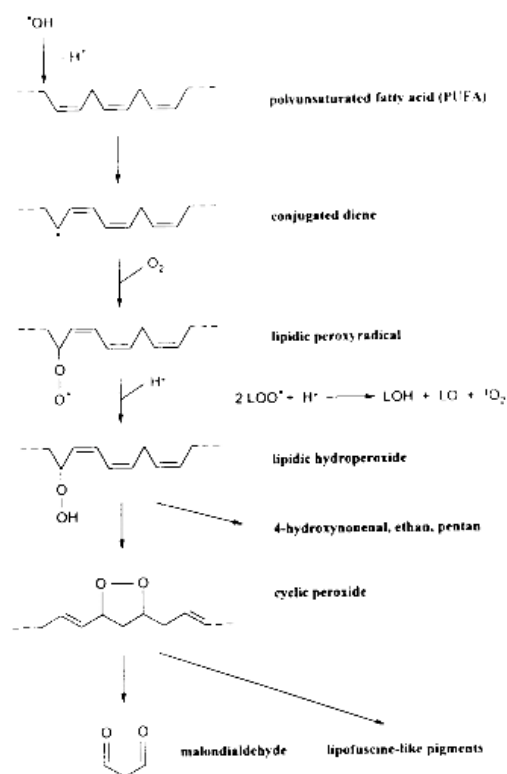
This phase begins with formation of the above mentioned reactive compounds, such as aldehydes or epoxides; the main products are malondialdehyde (MDA), 4-hydroxynonenal and hexanal. They can react with lysine residues of apoB<sub>100</sub>.

Reaction of aldehydes with amino groups of apoB<sub>100</sub> leads to the formation of so-called Schiff's bases exhibiting increased toxicity and risk for the organism.

Different methods can be used to appraise the damage due to lipid peroxidation (Esterbauer 1996). For

routine observations, we can use determinations of MDA, most often by a non-specific method such as thiobarbituric acid-reactive substances (TBARS). Further possible methods concern the determination of conjugated dienes or lipid hydroperoxides (spectrophotometrically or by gas chromatography) which precede the formation of aldehydes, or the estimation of pentane or ethane in expired air. Recently, immunological methods (RIA or ELISA) have been developed for exact determination of oxidatively modified proteins. The determination of 8-isoprostanes-2 $\alpha$  represents another promising method; commercial kits based on the immunochemical principle (ELISA) are available (Racek and Holeček 1999).

At the end of this oxidative process, modified LDL particles exhibit chemotactic, cytotoxic and immunogenic properties.



**Fig. 2.** Scheme of lipid peroxidation.

#### Chemotactic properties of oxidized LDL

Oxidized LDL attract neutrophilic granulocytes and monocytes into tissues; monocytes are converted to

macrophages (Griffith *et al.* 1988, Lugheed *et al.* 1991, Yla-Herttuala 1991, Maeba *et al.* 1995, Steinbrechers *et al.* 1989). Tissue macrophages then bind oxidized LDL to their scavenger receptors. This leads to uncontrolled intake of LDL-cholesterol and macrophages are transformed into so-called foam cells. These properties cannot be observed in native LDL.

#### *Cytotoxic properties of oxidized LDL*

Oxidized LDL display their harmful effects on cells by various mechanisms (Cathcart *et al.* 1989). They have a direct toxic effect on vessels, but the cause of cytotoxicity can also be the side- and end-products of LDL oxidation which are very reactive. Lesions of intima and smooth muscle cells can be observed due to the effect of malondialdehyde, 4-hydroxynonenal or other aldehydes. Oxidized LDL promote platelet aggregation and release of growth factors such as MCSF (mononuclear colony stimulating factor) and MCP-1

(monocyte chemotactic protein 1). Oxidized LDL cause changes of the endothelium leading to thrombosis. They also hinder vasodilatation. According to some authors there is a direct correlation between oxidized LDL and renal hypertension (Galle *et al.* 1995).

#### *Immunogenic properties of oxidized LDL*

According to a number of studies, there is evidence of strong immunogenicity of LDL particles modified by binding of malondialdehyde (MDA-LDL) or 4-hydroxynonenal (4-HNE-LDL) which are produced as lipid peroxidation end-products. These compounds bind to LDL and form epitopes leading to the production of specific autoantibodies (Bellomo *et al.* 1996, Jürgens *et al.* 1996, Karadi and Kostner 1990).

The biological properties of oxidized LDL particles are summarized in Table 1.

**Table 1.** Biological properties of oxidized LDL.

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Affinity to scavenger receptor of macrophages, source of intracellular accumulation of lipids

Chemotactic for plasma monocytes, macrophages and neutrophil leukocytes

Cytotoxic to endothelial and smooth muscle cells of vessel walls

Inhibition of prostaglandin I<sub>2</sub> synthesis

#### **Immunogenic – induction of IgoxLDL formation**

Promotion of platelet aggregation

Inhibition of nitric oxide synthase resulting in vasoconstrictor effect

Induction of different adhesive molecule synthesis

*In monocytes:* increased synthesis of interleukin 1 (IL-1), monocyte chemotactic protein 1 (MCP-1), monocyte colony stimulating factor (M-CSF), 15-lipoxygenase, lipoprotein lipase, platelet-derived growth factor (PDGF), scavenger receptors, metalloproteinases

*In macrophages:* increased synthesis of apolipoprotein E, lysophosphatidylcholine and cytokines

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#### ***Defense of LDL by antioxidants***

Antioxidants serve as a defense of LDL particles, which are especially susceptible to oxidative damage; they represent a primary defense of LDL against oxidation by free radicals. This defense can markedly retard the oxidation process. The principal representatives are  $\alpha$ -tocopherol,  $\beta$ -carotene and ubiquinol Q<sub>10</sub> (Lehr *et al.* 1995, Esterbauer *et al.* 1996a,b,c). Their role is important above all during the lag phase, when they hinder rapid oxidation. This leads to their consumption, if they are not effectively regenerated by antioxidants in the hydrophilic compartment, especially by ascorbic acid. A regular intake of vitamin E and other antioxidants can thus retard oxidative modification of LDL.

#### ***Physiology and biochemistry of IgoxLDL***

The mechanism of IgoxLDL formation is not clear as yet. On the other hand, it is certain that oxidative damage to LDL is the basic presumption of production of these antibodies. Oxidized LDL express a great number of epitopes and cause production of polyclonal autoantibodies against these products, especially against apoB<sub>100</sub> modified by these products, namely by binding malondialdehyde and 4-hydroxynonenal (Galle *et al.* 1995, Jürgens *et al.* 1996, Lehr *et al.* 1995, Waeg *et al.* 1996). IgoxLDL can be demonstrated either directly in intimal lesions or as a component of circulating immune complexes.

The properties and significance of IgoxLDL has not yet been clarified. It is known that they are produced during changes of lipoprotein structure but they can also be identified in healthy subjects; this finding complicates the whole matter. IgoxLDL bind to modified LDL so that the following three variants can occur:

a) Metabolism of LDL is retarded and hyperlipidemia is potentiated. This can be explained by binding IgoxLDL directly to LDL and the subsequent hindrance of LDL intake by cells (Morganelli *et al.* 1995). This process takes place probably at the level of macrophages.

b) Antibodies can be bound to the lipase receptor of LDL; lipase is then unable to split lipids in these particles.

c) IgoxLDL are bound directly to lipase and block lipolysis. This mechanism would explain some cases of hyperlipidemia which can be influenced only with difficulty, e.g. coronary artery disease or eclampsia (Morganelli *et al.* 1995).

### Classification of IgoxLDL

IgoxLDL do not form a homogeneous group but a varied mixture of antibodies which were divided by Riesen and Nosedá (1975) on the basis of immunoglobulin groups or according to their manifestation.

### Isoantibodies caused by HDL and LDL polymorphism

They were discovered for the first time in subjects after repeated blood transfusions or as an accompanying phenomenon of thalassemia.

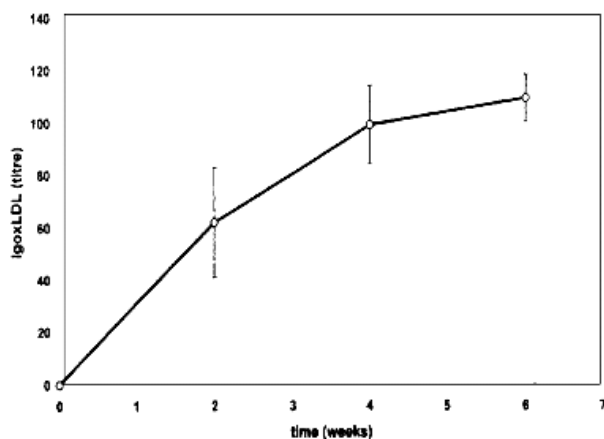
### Antibodies against lipid phase of LDL

These are autoantibodies found either in patients with multiple myeloma or as polyclonal autoantibodies in patients with essential (familial) hyperlipidemia. In the case of myeloma the equilibrium is also shifted towards hyperlipidemia which can be caused by a decrease in lipolysis due to the above mentioned presence of autoantibodies. Phospholipids or rather phospholipids modified by oxidation are anticipated to be antigenic determinants in this case (Mizutani *et al.* 1995).

### Antibodies against modified apoB<sub>100</sub>

This group of IgoxLDL is linked to an increased risk of atherosclerosis (Maeba *et al.* 1995), as well as a high concentration of apoB<sub>100</sub> itself. It is usually found in patients with familiar hypercholesterolemia which is the cause of premature atherosclerosis and other degenerative vascular changes. These antibodies are mono- or polyclonal in nature; they are also present in some cases of M-proteinemia, in systemic and rheumatic diseases and breast cancer. This class of IgoxLDL is also accompanied by hyperlipidemia.

Another criterion of division of IgoxLDL is based on the immunoglobulin class: IgA or IgG autoantibodies can be distinguished (Beaumont 1965, Beaumont *et al.* 1988). IgAoxLDL react relatively non-specifically with LDL and HDL of different animal species while IgGoxLDL react exclusively with human lipoproteins.



**Fig. 3.** Titers of IgoxLDL in starved dogs and during their realimentation; results as means  $\pm$  S.D. (according to Tatzber *et al.* 1997).

Tatzber *et al.* (1997) studied dogs during starvation and during their gradual recovery. During the first two weeks IgoxLDL were not demonstrable, but after re-introduction of the standard diet, the animals recovered and IgoxLDL titer slowly rose. It ultimately reached normal values observed in healthy animals (see also Fig. 3). Similar results were found in septic patients (Tatzber and Esterbauer 1995) and patients after liver and heart transplantation (Khoshsorur *et al.* 1996). In cases of recovery IgoxLDL rose, but in fatal cases their level remained low. It seems that the production of IgoxLDL can rise during convalescence after severe diseases.

### **IgoxLDL and atherosclerosis**

Salonen *et al.* (1992) measured and compared the diameter of carotid artery, levels of blood lipids, smoking habits and IgoxLDL titer in patients with atherosclerosis and in healthy persons in their retrospective study. They found a positive correlation between an increase in carotid intima thickness, LDL concentration in the blood serum and an increase of IgoxLDL titer. This finding confirms the significance of IgoxLDL in atherosclerosis development. Nevertheless, there were some doubts as to whether a single sonographic examination of carotid arteries can provide information about the patient's state and the reproducibility of the results was also discussed. Other experts appreciated the study as a positive stimulus for prevention of atherosclerosis (in accordance with the therapeutic decrease of cholesterol levels) and explanation of the role of antioxidants. The phenomenon of IgoxLDL was also observed in other diseases caused by atherosclerosis. Nevertheless, Salonen *et al.* (1992) were the first authors who mentioned the possible relation between atherosclerosis and immune reactions. Karachava *et al.* (1993) speak about the atherogenic potential of some serum samples with a high titer of IgoxLDL.

It was generally observed in experimental atherosclerosis that a high incidence of vascular diseases occurs in parallel with increased titer of IgoxLDL, independently on LDL levels. After myocardial infarction, a temporary decrease of IgoxLDL level was observed, which increased again in the convalescence phase (Vrkic *et al.* 1977). It can be explained by a massive release of free radicals during acute myocardial infarction and the following increase of lipid peroxidation. IgoxLDL hinder further damage to vessels and that is why the level of autoantibodies temporarily

decreases (Schumacher *et al.* 1995). Similar observations were made in patients after injuries and with cerebrovascular ischemia (Borovic *et al.* 1995, Golod and Balanova 1976). A high titer of antibodies against oxidized LDL was also observed in other diseases with hypertension (for example preeclampsia). A positive correlation between atherosclerosis and the IgoxLDL titer was confirmed in many studies (Decarvalho 1982, Klimov *et al.* 1982, Lopes-Virella and Virella 1994).

Antibodies against oxLDL were found in many other diseases such as diabetes mellitus or the renovascular syndrome and uremia. Smoking together with hypercholesterolemia also increase the IgoxLDL titer (Salonen *et al.* 1992, Heitzer *et al.* 1996). Primary chronic polyarthritis, rheumatic fever, morbus Bechtjerev or lupus erythematoses are other diseases often linked with increased IgoxLDL levels. Hyperlipidemia and IgoxLDL directed mostly against apoB<sub>100</sub> is another interesting finding (Hansen *et al.* 1995, Savolainen *et al.* 1995).

Steinerová *et al.* (1999) found practically the same level of IgoxLDL in newborns as in their mothers; these values did not differ from those in the healthy population of non-pregnant women of the same age. A significant increase of Igox LDL titer in the same newborns during the first three months after birth (60 % of those children had IgoxLDL higher than 1800 U/l, the value which was never found in any of the adult subjects) confirms the presence of oxidative stress during labor and in the early period after birth and the reaction of the organism to LDL oxidation. The level of lipid peroxidation is similar in the mother and her neonate with regard to the blood MDA levels. Enzymes of the antioxidant defense system – superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase – rapidly develop in the late gestation period and human erythrocyte SOD development is not fully completed at birth since its activity is lower in comparison with maternal levels (Štípek *et al.* 1995). Lapin *et al.* (1996) found higher IgoxLDL levels in young people in comparison with older persons. As a higher load of free radicals can be assumed in elderly subjects, the decrease of IgoxLDL titer with age can be considered as an expression of their protective function.

Steinerová *et al.* (1996) described a decrease in IgoxLDL titer after three months' administration of both fenofibrate and vitamin E. Thus a decreased concentration of oxidized LDL might reflect a lower loading of the organism with free radicals. The decrease

in IgGoxLDL titer was very slow and lasted many months. For this reason, this parameter cannot be considered suitable for describing rapid changes during oxidative stress of the organism.

Vaarala *et al.* (1995) reported cross-reactivity between IgGoxLDL and antiphospholipid autoantibodies as a sign of a possible immunological link between thrombotic and atherosclerotic processes. Savolainen *et al.* (1995) described a positive correlation between IgGoxLDL and antiphospholipid antibodies in patients with juvenile chronic arthritis. A high degree of correlation ( $r=0.79$ ,  $p<0.001$ ) was found by Mizutani *et al.* (1995) in a species of mice with a tendency to develop autoimmune diseases. The cross-reactivity between IgGoxLDL and antiphospholipid antibodies can be explained according to Horkko *et al.* (1996) by the origin of antiphospholipid antibodies as a reaction of the organism to oxidatively modified phospholipids, especially of cardiolipin, since the affinity of monoclonal antibodies against oxidized LDL to cardiolipin markedly rises with its oxidation. Zima *et al.* (1998b) observed a positive correlation between anticardiolipin antibodies and IgGoxLDL in hemodialysis patients who had increased concentrations of apoB<sub>100</sub>, the major protein in LDL regulating cholesterol synthesis and metabolism. On the contrary, unchanged levels of IgGoxLDL in hemodialysis patients may be caused by impaired immunological reactivity of these persons. The production of autoantibodies depends on the "quality" of the immune system and the age of subjects.

IgGoxLDL correlates with other parameters related to the atherosclerotic process, e.g. homocysteine levels in patients with mild hyperhomocysteinemia ( $p<0.05$ ). Anti- $\beta$ -2-glycoprotein I IgG also positively correlates ( $r=0.413$ ,  $p<0.0001$ ) in groups of nephrological patients (Wegener's granulomatosis, systemic lupus erythematoses, IgA nephropathy, nephrotic syndrome). IgGoxLDL was increased in patients with autosomally dominant polycystic kidney disease, lupus nephritis and Wegener's granulomatosis in which plasma exchange treatment decreased their titer (Zima *et al.* 1997a, Fialová *et al.* 1999).

With regard to the occurrence of IgGoxLDL in healthy persons and a very broad range of reference values (395–605 U/l), Zima *et al.* (1997b) do not consider this examination as a parameter suitable for determining the possible risk of atherosclerosis and oxidative stress. Paiker *et al.* (2000) expressed the same opinion in patients with familiar hypercholesterolemia in which IgGoxLDL titers cannot also be used as a predictive marker of the presence or severity of atherosclerosis.

As a result of the repeatedly demonstrated positive correlation with antiphospholipid and other antibodies, it is possible to use IgGoxLDL as a marker for describing the total production of autoantibodies in various diseases. The changes in concentrations and correlations of IgGoxLDL, anti- $\beta$ -2-glycoprotein I IgG and antiphospho-lipid antibodies support the immunological link between thrombotic and atherosclerotic processes in the human body.

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