

Lipoprotein receptors in arterial tissue: relation to the pathology of atherosclerosis

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Coronary Artery Disease 1994, 5:211-215

Keywords: lipoproteins, low-density lipoprotein receptors, low-density lipoprotein receptor-related protein, scavenger receptors

Excessive intracellular deposition of cholesterol and cholesterol esters is a distinctive feature of all stages of the evolution of atherosclerotic lesions. Lipid deposition is a well known characteristic of macrophages, but is also a feature of smooth muscle cells and endothelium. The source of the intracellular deposits of cholesterol is commonly considered to be plasma-derived lipoproteins. Thus, the mode of recognition of these lipid-containing particles on cell surfaces could be a critical factor modulating the atherogenic process. It is at present assumed that the major portion of lipoprotein entry into cells of the evolving atherosclerotic lesion is mediated via specific surface receptors. This brief review will focus on the role of lipoprotein receptors at the level of the arterial wall rather than those controlling whole-body cholesterol homeostasis. Accordingly, the receptor populations that may contribute to the manifestations of vascular pathology and the evidence for a role of the receptors *in vivo* will be discussed. The major properties of these lipoprotein receptors are summarized in Table 1.

Low-density lipoprotein receptors

The definition of low-density lipoprotein (LDL) receptors was a classical event in cell biology because it was the first demonstration of a specific receptor mediating the intracellular delivery of a macromolecule. The role of LDL receptors in the development of atherosclerosis can be dramatically seen in individuals afflicted with familial hypercholesterolemia [1]. Deficiency of LDL receptors leads to gross hypercholesterolemia and rapid production of atherosclerosis. Indeed, a rabbit species genetically

deficient in functional LDL receptors, the Watanabe heritable hyperlipidemic rabbit, is a commonly used animal model of atherosclerosis. There is therefore no doubt that LDL receptor deficiency leads to an imbalance in whole-body cholesterol homeostasis.

Although LDL receptors have major effects on plasma cholesterol concentrations, their effects at the level of the vessel wall are uncertain. Passage of LDL through an intact endothelium monolayer *in vivo* does not require the presence of LDL receptors [2]. Once in the subendothelial space, however, this region has the highest catabolic rate for LDL of any area in the body when data are normalized to tissue mass [3]. This high catabolic rate of LDL in the intima of normolipidemic rabbits has been attributed to an LDL receptor-dependent mechanism. There is little information on the role of LDL receptors in evolving atherosclerotic lesions. On the basis of the sterol regulatory components of the promoter region of LDL receptor gene, it may be anticipated that LDL receptors are rapidly downregulated in evolving atherosclerotic tissue. The fact that familial hypercholesterolemic patients exhibit florid foam cell formation in the absence of LDL receptors may be evidence that LDL receptors have no function in the disease process at the level of the arterial wall. However, other than the hypercholesterolemic effects of LDL-receptor deficiency, there is paucity of information defining the role of LDL receptors in the loci of the vessel wall.

Scavenger receptors

The demonstration that native LDL could not promote excessive intracellular lipid deposition led to

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Table 1. Characteristics of lipoprotein receptors.

Receptor	Ligands	Factors promoting upregulation	Factors promoting downregulation	Receptor-positive cells in vascular tissue	Possible functions in atherosclerotic lesions
LDL receptors	Apolipoprotein B Apolipoprotein E	Cholesterol starvation	Cholesterol excess	Monocytes Endothelial smooth muscle T-lymphocytes	Unknown
Scavenger receptors	AcLDL MDALDL Oxidized LDL LPS	GM-CSF PDGF	LPS TNF- α TGF- β IFN- γ	Macrophages Endothelial smooth muscle Fibroblasts	Cholesterol deposition Monocyte endothelial adhesion
LDL receptor-related protein	Apolipoprotein-E-enriched β -VLDL Activated α_2 -macroglobulin t-PA UR-PAI-1 complexes Pseudomonas endotoxin A	Insulin	IFN- γ Cyclic AMP	Macrophages Fibroblasts	Cholesterol deposition Extracellular matrix modelling
Fc γ RII-B2	Oxidized LDL		Macrophage activation	Macrophages	Unknown
CD36 (glycoprotein IV; GpIIIb)	Moderately and extensively oxidized LDL	IFN- γ	Protein kinase C agonists	Macrophages Platelets Endothelium	Intracellular cholesterol deposition Collagen binding Monocyte-platelet binding

LDL, low-density lipoprotein; AcLDL, acetylated low-density lipoprotein; GM-CSF, granulocyte-macrophage colony stimulating factor; LPS, lipopolysaccharide; MDALDL, malondialdehyde-LDL; PDGF, platelet-derived growth factor; TNF, tumor necrosis factor; TGF, transforming growth factor; IFN- γ , interferon- γ ; VLDL, very low-density lipoprotein; t-PA, tissue plasminogen activator; UR-PAI-1, urokinase-plasminogen activator inhibitor-1 complex; CD, complex of differentiation.

the proposal that LDL was 'damaged' as a result of its long residence time and that the modified material leads to foam cell formation. The receptor mediating the interaction of this damaged material was termed the 'scavenger' receptor. Scavenger receptors were initially characterized using acetylated LDL, but further studies have demonstrated that numerous ligands interact with this receptor. These ligands include oxidized lipoproteins and proteins, specific polynucleotides, polysaccharides, anionic phospholipids, and several miscellaneous compounds such as endotoxin, polyvinyl sulphate, and asbestos.

Scavenger receptor activity was originally described in cultured macrophages. The scavenger receptor protein was first characterized in the monocyte cell line THP-1 after differentiation into macrophages with phorbol ester treatment [4]. Scavenger receptor protein was subsequently isolated from bovine liver [5]. The recent cloning of scavenger receptors by Krieger and colleagues [6,7] has revealed that scavenger receptors exist in two forms, termed type I and II, which are alternatively spliced products of the same gene. The structure of scavenger receptors has recently been reviewed [8].

In addition to macrophages, scavenger receptor activity is present in endothelial cells [9], smooth muscle cells, and fibroblasts [10]. These cell culture studies suggest that scavenger receptor activity could be

present on most of the major cell types present in atherosclerotic lesions. Although scavenger receptor activity has consistently been noted in endothelial cells, this receptor is structurally distinct from the gene cloned by Kodama *et al.* [7] because molecular probes derived from scavenger receptor cDNA do not react with mRNA from endothelial cells [11]. It should be noted that lipid engorgement of endothelium occurs in regions of evolving atherosclerotic lesions [12], although the receptor mediating this effect has not been elucidated.

Scavenger receptors are not regulated by intracellular cholesterol content, even in massively engorged foam cells [13], but may be regulated by many cytokines. For example, lymphokines downregulate macrophage scavenger receptor activity [14]. In addition, interferon- γ , transforming growth factor- β , and tissue necrosis factor- α decrease scavenger receptor activity. Upregulation is achieved in the presence of colony stimulating factor in macrophages. In smooth muscle cells, receptor activity is increased in the presence of platelet-derived growth factor. Although these regulatory mechanisms operate in cultured cells, the importance of this level of regulation *in vivo* is not known. However, T-lymphocytes are present in specific regions of atherosclerotic lesions in large numbers (as discussed by Hansson and Stemme in this issue), which

could impart local regulatory mechanisms for scavenger receptors.

Despite the many studies demonstrating scavenger receptor activity in cultured cells, the role of scavenger receptors in atherogenic events *in vivo* is poorly defined. Scavenger receptor protein and mRNA have been detected in human atherosclerotic tissue [15,16]; however, no systematic evaluation of the function of scavenger receptors in evolving atherosclerotic lesions has been carried out *in vivo*. Indeed, there is reason to believe that oxidative modification of lipoproteins in the vessel wall (see the review by Heinecke in this issue) does not occur to a sufficient extent to permit interaction via scavenger receptors [17,18].

The effect of the modulation of scavenger receptor activity on atherogenesis is uncertain. The presence of scavenger receptors may assist the atherogenic process by promoting ingestion of lipid and formation of foam cells. The absence of scavenger receptors, however, may prevent removal of extracellular lipids; the subsequent increase in extracellular lipids could lead to cell toxicity. Furthermore, this increase in extracellular lipids may trigger an increase in the phagocytic removal of lipids. It has recently been shown that the formation of foam cells via a phagocytic pathway may lead to lysosomal accumulation of esterified and unesterified cholesterol. This lysosomal accumulation may not be amenable to removal in the same manner in which cytosolic depositions of lipid are after entry via scavenger receptors [19]. The availability of genetically modified mice in which scavenger receptor activity is either enhanced or ablated will provide insight into the role of scavenger receptors in the atherogenic process.

Low-density lipoprotein receptor-related protein/ α_2 -macroglobulin receptor

While screening for cysteine-rich complement proteins, Herz *et al.* [20] cloned a protein from a human liver library that had striking structural resemblances to low-density lipoprotein receptors, hence the name 'low-density lipoprotein receptor-related protein' (LRP). LRP incorporates tandem repeats of the LDL receptor. Despite the structural similarity to LDL-receptors, LRP does not bind LDL. Its binding of apolipoprotein-E liposomes and apolipoprotein-E-enriched beta very low-density lipoprotein has led to the suggestion that LRP is a chylomicron remnant receptor [21]. However, there is no uniform agreement on this assignment as a chylomicron remnant receptor [22].

Subsequent studies [23] demonstrated that LRP and the α_2 -macroglobulin receptor are the same protein. LRP/ α_2 -MR has an extraordinarily broad ligand specificity that includes not only the previously mentioned lipoprotein ligands, but also tissue plas-

minogen activator [24], urokinase-plasminogen activator inhibitor-1 complexes [25], lipoprotein (a) [26], and lipoprotein lipase [27]. In addition, since activated α_2 -macroglobulin can form complexes with many proteins, this receptor may act as a clearance mechanism for proteins of diverse functions that include several cytokines.

Relatively little information is available regarding factors modulating LRP. Unlike LDL receptors, no sterol regulatory elements exist in the 5' promoter region, and consequently cholesterol does not influence receptor activity [28]. Recently, LaMarre *et al.* [29] demonstrated that interferon- γ decreased the activity of LRP/ α_2 -MR in cultured macrophages, but tumor necrosis factor- α , transforming growth factor- β 1, and interleukin-6 had no effect. Thus, the presence of lymphocytes within atherosclerotic lesions could act to provide local regulation of both LRP/ α_2 -MR and scavenger receptors.

Information on the presence of LRP/ α_2 -MR in vascular tissue during the evolution of atherosclerosis is limited. In preliminary studies (Daugherty and Rateri, submitted for publication), we have demonstrated increased LRP/ α_2 -MR mRNA in atherosclerotic tissue, where the protein is predominantly located in macrophages. Given the wide range of ligands capable of interacting with LRP/ α_2 -MR, it appears likely that this receptor will have an important role in the atherogenic process. As a result of the diversity of LRP-mediated interactions, as with scavenger receptors, it cannot readily be predicted whether the progression of atherogenesis would be facilitated by the presence or absence of this receptor class.

Other lipoprotein receptor classes

With the current emphasis on the role of lipoprotein oxidation as a pivotal mechanism in the development of atherosclerotic lesions, there is intense interest in determining whether scavenger receptors are the only receptor class interacting with these particles. Recently, two further classes of lipoprotein receptors have been shown to interact with oxidized LDL. Expression cloning experiments determined that oxidized LDL interacted with Fc γ RII-B2 receptors [30]. This receptor does not interact with acetylated LDL. A neutralizing antibody inhibited the interaction of oxidized LDL with cells transiently expressing Fc γ RII-B2 receptors, but failed to inhibit the metabolism of oxidized LDL by macrophages. The physiological relevance of this receptor is therefore unknown at present. CD36 was also observed to interact with oxidized LDL, but not acetylated LDL, through expression cloning techniques [31]. CD36 is also known as glycoprotein IV and glycoprotein IIIb. In contrast to Fc γ RII-B2, a neutralizing anti-CD36 antibody inhibited interactions with oxidized LDL both in cells transfected with CD36 and

in macrophages. Furthermore, the extent of LDL oxidation needed to initiate interactions with CD36 is much more modest than that needed to allow interaction with either scavenger receptors or Fcγ RII-B2 receptors. As noted earlier, it is uncertain whether LDL is sufficiently modified *in vivo* to allow interactions with scavenger receptors. Although there is no evidence that these two receptor classes exist in atherosclerotic lesions, these initial observations provide a solid rationale for further investigation.

Summary

The molecular and cellular physiology of several classes of lipoprotein receptors has been extensively characterized *in vitro*. However, the evidence that these lipoprotein receptors mediate the morphological events characteristic of atherosclerotic development *in vivo* is limited. The increasing availability of reagents to characterize receptor mRNA and protein will enable the definition of receptors involved in atherosclerotic lesion progression. These studies should set the stage for the more difficult task of modulating lipoprotein receptors to determine whether their activity is a critical component of atherogenesis.

Annotated references and recommended reading

- Of special interest
- Of outstanding interest

1. Goldstein JL, Brown MS: Binding and degradation of low density lipoproteins by cultured human fibroblasts. Comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. *J Biol Chem* 1974, 249:153-162.

A classic paper that provided the first description of the LDL receptor pathway in cultured cells.

2. Wiklund O, Carew TE, Steinberg D: Role of the low density lipoprotein receptor in penetration of low density lipoprotein into rabbit aortic wall. *Arteriosclerosis* 1985, 5:135-141.
3. Carew TE, Pittman RC, Marchand ER, Steinberg D: Measurement *in vivo* of irreversible degradation of low density lipoprotein in the rabbit aorta. Predominance of intimal degradation. *Arteriosclerosis* 1984, 4:214-224.
4. Via DP, Dresel HA, Cheng S, Gotto AM: Murine macrophage tumors are a source of a 260,000 Dalton acetyl-low density lipoprotein receptor. *J Biol Chem* 1985, 260:7379-7386.
5. Kodama T, Reddy P, Kishimoto C, Krieger M: Purification and characterization of a bovine acetyl low density lipoprotein receptor. *Proc Natl Acad Sci USA* 1988, 85:9238-9242.
6. Kodama T, Freeman M, Rohrer L, Zabrecky J, Matsudaira P, Krieger M: Type I macrophage scavenger receptor contains alpha-helical and collagen-like coiled cells. *Nature* 1990, 343:531-535.

Report of the first genetic sequence for scavenger receptors, which provided information to predict the protein structure.

7. Rohrer L, Freeman M, Kodama T, Penman M, Krieger M: Coiled-coil fibrous domains mediate ligand binding by macrophage scavenger receptor type II. *Nature* 1990, 343:570-572.
8. Krieger M, Acton S, Ashkenas J, Pearson A, Penman M, Resnick D: Molecular flypaper, host defense, and atherosclerosis — structure, binding properties, and functions of macrophage scavenger receptors. *J Biol Chem* 1993, 268:4569-4572.

A review of the diverse functions that have been ascribed to the scavenger receptor.

9. Baker DP, van Lenten B, Fogelman AM, Edwards PA, Kean C, Berliner JA: LDL, scavenger, and beta-VLDL receptors on aortic endothelial cells. *Arteriosclerosis* 1984, 4:248-255.
 10. Pitas RE: Expression of the acetyl low density lipoprotein receptor by rabbit fibroblasts and smooth muscle cells — up-regulation by phorbol esters. *J Biol Chem* 1990, 265:12722-12727.
 11. Bickel PE, Freeman MW: Rabbit aortic smooth muscle cells express inducible macrophage scavenger receptor messenger RNA that is absent from endothelial cells. *J Clin Invest* 1992, 90:1450-1457.
 12. Rosenfeld ME, Tsukada T, Gown AM, Ross R: Fatty streak initiation in Watanabe heritable hyperlipidemic and comparably hypercholesterolemic fat-fed rabbits. *Arteriosclerosis* 1987, 7:9-23.
 13. Rosenfeld ME, Khoo JC, Miller E, Parthasarathy S, Palinski W, Witztum JL: Macrophage-derived foam cells freshly isolated from rabbit atherosclerotic lesions degrade modified lipoproteins, promote oxidation of low-density lipoproteins, and contain oxidation-specific lipid-protein adducts. *J Clin Invest* 1991, 87:90-99.
 14. Fogelman AM, Seager J, Haberland ME, Hokom M, Tanaka R, Edwards PA: Lymphocyte-conditioned medium protects human monocyte-macrophages from cholesteryl ester accumulation. *Proc Natl Acad Sci USA* 1982, 79:922-926.
 15. Matsumoto A, Naito M, Itakura H, Ikemoto S, Asaoka H, Hayakawa I, et al.: Human macrophage scavenger receptors — primary structure, expression, and localization in atherosclerotic lesions. *Proc Natl Acad Sci USA* 1990, 87:9133-9137.
 16. Yla-Herttuala S, Rosenfeld ME, Parthasarathy S, Sigal E, Sarkioja T, Witztum JL, Steinberg D: Gene expression in macrophage-rich human atherosclerotic lesions — 15-lipoxygenase and acetyl low density lipoprotein receptor messenger RNA colocalize with oxidation specific lipid-protein adducts. *J Clin Invest* 1991, 87:1146-1152.
 17. Daugherty A, Zweifel BS, Sobel BE, Schonfeld G: Isolation of low density lipoprotein from atherosclerotic vascular tissue of Watanabe heritable hyperlipidemic rabbits. *Arteriosclerosis* 1988, 8:768-777.
 18. Steinbrecher UP, Lougheed M: Scavenger receptor-independent stimulation of cholesterol esterification in macrophages by low density lipoprotein extracted from human aortic intima. *Arteriosclerosis and Thrombosis* 1992, 12:608-625.
 19. Brown MS, Ho YK, Goldstein JL: The cholesterol ester cycle in macrophage foam cells. Continual hydrolysis and re-esterification of cytoplasmic cholesteryl esters. *J Biol Chem* 1980, 255:9344-9352.
 20. Herz J, Hamann U, Rogne S, Myklebost O, Gausepohl H, Stanley KK: Surface location and high affinity for calcium of a 500-kd liver membrane protein closely related to the LDL-receptor suggest a physiological role as lipoprotein receptor. *EMBO J* 1988, 7:4119-4127.
- A paper describing the sequencing and characterization of a large macromolecule, known as LRP, which has structural similarities with the LDL receptor.
21. Beisiegel U, Weber W, Ihrke G, Herz J, Stanley KK: The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature* 1989, 341:162-164.

22. Huettinger M, Retzek H, Hermann M, Goldenberg H: **Lactoferrin specifically inhibits endocytosis of chylomicron remnants but not alpha-macroglobulin.** *J Biol Chem* 1992, 267:18551-18557.
23. Kristensen T, Moestrup SK, Gliemann J, Bendtsen L, Sand O, Sottrup-Jensen L: **Evidence that the newly cloned low-density-lipoprotein receptor related protein (LRP) is the alpha-2-macroglobulin receptor.** *FEBS Lett* 1990, 276:151-155.
24. Bu GJ, Williams S, Strickland DK, Schwartz AL: **Low density lipoprotein receptor-related protein/alpha2-macroglobulin receptor is an hepatic receptor for tissue-type plasminogen activator.** *Proc Natl Acad Sci USA* 1992, 89:7427-7431.
25. Orth K, Madison EL, Gething MJ, Sambrook JF, Herz J: **Complexes of tissue-type plasminogen activator and its serpin inhibitor plasminogen-activator inhibitor type-1 are internalized by means of the low density lipoprotein receptor-related protein/alpha2-macroglobulin receptor.** *Proc Natl Acad Sci USA* 1992, 89:7422-7426.
26. Marz W, Beckmann A, Scharnagl H, Siekmeier R, Mondorf U, Held I, et al.: **Heterogeneous lipoprotein(a) size isoforms differ by their interaction with the low density lipoprotein receptor and the low density lipoprotein receptor-related protein/alpha(2)-macroglobulin receptor.** *FEBS Lett* 1993, 325:271-275.
27. Chappell DA, Fry GL, Waknitz MA, Muhonen LE, Pladet MW, Iverius PH, Strickland DK: **Lipoprotein lipase induces catabolism of normal triglyceride-rich lipoproteins via the low density lipoprotein receptor-related protein/alpha-2-macroglobulin receptor *in vitro* — A process facilitated by cell-surface proteoglycans.** *J Biol Chem* 1993, 268:14168-14175.
28. Kutt H, Herz J, Stanley KK: **Structure of the low density lipoprotein receptor protein (LRP) promoter.** *Biochim Biophys Acta* 1989, 1009:229-236.
29. LaMarre J, Wolf BB, Kittler ELW, Quesenberry PJ, Gonias SL: **Regulation of macrophage alpha2-macroglobulin receptor/low density lipoprotein receptor-related protein by lipopolysaccharide and interferon-gamma.** *J Clin Invest* 1993, 91:1219-1224.
30. Stanton LW, White RT, Bryant CM, Protter AA, Endemann G: **A macrophage Fc receptor for IgG is also a receptor for oxidized low density lipoprotein.** *J Biol Chem* 1992, 267:22446-22451.
31. Endemann G, Stanton LW, Madden KS, Bryant CM, White RT, Protter AA: **CD36 is a receptor for oxidized low density lipoprotein.** *J Biol Chem* 1993, 268:11811-11816.

This study characterized an alternative mechanism by which mildly oxidized forms of lipoproteins can interact with macrophages through a scavenger receptor-independent mechanism.

Immune mechanisms in atherosclerosis

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Coronary Artery Disease 1994, 5:216–222

Keywords: atherosclerosis, T lymphocytes, cytokines, antigens

Atherosclerosis represents one of the main causes of death and disease in industrialized societies. Pathogenetically, it is characterized by a focal, slow, and progressive accumulation of cells, extracellular matrix, and lipid in the intima of medium-sized and large arteries. The resulting luminal occlusion and thrombosis lead to fatal or severely disabling clinical manifestations such as myocardial and cerebral infarction.

Large epidemiological studies have provided important information on atherosclerosis, which has been used in attempts to prevent the disease. It has been estimated, however, that only approximately 50% of the incidence of cardiovascular disease can be explained by the major risk factors. Consequently, there is room for substantial, as yet unknown, contributing factors. Furthermore, epidemiology has not provided an understanding of the pathophysiological mechanisms of atherosclerosis. With the exception of a small number of genetic disorders of lipid metabolism, the cause and molecular and cellular pathogenesis of atherosclerosis remain unclear.

The growing realization of the presence of T lymphocytes in the plaque [1] and the finding of antibody responses to plaque constituents has reawakened the interest in inflammatory and immune components in atherogenesis. Much of the current interest in atherosclerosis research is focused on cell recruitment and cellular communication in the evolving atherosclerotic plaque. The presence of activated T cells and signs of T cell cytokine secretion in atherosclerotic lesions suggest a role for local T cell responses in atherogenesis. Several studies demonstrating the presence of autoantibodies to modified lipoproteins or heat shock proteins indicate that atherosclerosis may also involve B cell responses. The present review aims to summarize and discuss recent progress in this area.

Recruitment of lymphocytes and monocytes

Of the potential adhesion molecules mediating T cell binding to endothelium, intercellular adhesion molecule-1 (ICAM-1), E-selectin, and vascular cell adhesion molecule-1 (VCAM-1) have been detected in atherosclerotic plaques [2,3]. Of special interest is the discovery of VCAM-1 expression early in experimental atherosclerosis, before macrophages appear in the subendothelium [4,5], and in advanced human lesions [6]. In contrast to ICAM-1 and E-selectin, which bind granulocytes as well as mononuclear cells, VCAM-1 binds only monocytes and lymphocytes [7,8]. This may explain the mononuclear infiltrate in atherosclerosis. Furthermore, lysophosphatidylcholine, which may be generated during lipoprotein oxidation in atherosclerotic plaques, selectively induces the expression of VCAM-1 in cultured human endothelial cells [9]. When the blood-borne cell, through adhesive interactions, has entered the intimal space, further proliferation, migration, and metabolism may be regulated by intercellular signal substances and cell-matrix interactions.

Cytokines in atherosclerotic lesions

The earliest morphological evidence of disease in hypercholesterolemic animal models is the attachment of monocytes to the intact endothelium [10,11,12]. This is followed by gradual subendothelial accumulation of macrophages, which turn into lipid-laden foam cells [10,11,12], and of T lymphocytes [13]. This stage is also recognized in early human lesions [14,15,16]. In this process, smooth muscle cells (SMCs) migrate from the media into the intima, proliferate, and deposit extracellular matrix

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