Dossier: Aging and age-related diseases

Extracellular matrix remodeling and matrix metalloproteinases in the vascular wall during aging and in pathological conditions

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Abstract

The extracellular matrix provides a structural framework essential for the functional properties of vessel walls. The three dimensional organization of the extracellular matrix molecules — elastin, collagens, proteoglycans and structural glycoproteins — synthesized during fetal development — is optimal for these functions. In uninjured arteries and veins, some proteases are constitutively expressed, but through the control of their activation and/or their inhibition by inhibitors, these proteases have a very low activity and the turnover of elastic and collagen fibers is low. During aging and during the occurrence of vascular pathologies, the balance between proteases and their inhibitors is temporally destroyed through the induction of matrix metalloproteinase gene expression, the activation of zymogens or the secretion of enzymes by inflammatory cells. Smooth muscle cells, the most numerous cells in vascular walls, have a high ability to respond to injury through their ability to synthesize extracellular matrix molecules and protease inhibitors. However, the three dimensional organization of the newly synthesized extracellular matrix is never functionally optimal. In some other pathologies — aneurysm — the injury overcomes the responsive capacity of smooth muscle cells and the quantity of extracellular matrix decreases. In conclusion, care should be taken to maintain the vascular extracellular matrix reserve and any therapeutic manipulation of the protease/inhibitor balance must be perfectly controlled, because an accumulation of abnormal extracellular matrix may have unforeseen adverse effects.

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1. The extracellular matrix of the vascular wall

The cells of the three layers of the vascular wall, intima, media and adventitia, lie on or are embedded in their extracellular matrix (ECM). In the intima, endothelial cells lie on their basement membrane (Table 1). This basement membrane contains type IV collagen associated with laminins and perlecan. Small and large arteries, as well as veins, have an internal elastic lamina between the intima and the media. The media of the aorta and the other elastic arteries contains several other elastic laminae. Between these elastic laminae, smooth muscle cells and other ECM components (collagen fibers, structural glycoproteins, proteoglycans (PGs)) are present. The PGs in the media are hyaluronan, decorin, versican and perlecan. The adventitia mainly contains collagen fibers and fibroblasts.

Collagens and elastin are the main protein constituents of vessels. The biomechanical properties of vessels, particularly of the major arteries and veins, are largely dependent on the absolute and relative quantities of these two constituents [54].

Among the 26 different collagen types described until now, type I and III collagens are the major fibrillar collagens detectable in vessels, representing 60% and 30% of vascular collagens, respectively [42,50,60]. The remaining 10% include fibrillar type V collagen, types XII and XIV collagen,
which are fibril-associated collagens with interrupted triple helix (FACITs), type VI microfibrillar collagen, basement membrane type IV collagen and type VIII collagen. The basic unit of fibrillar collagens is the 300 nm triple helix, with each chain comprising about 330 repeats of the amino acid sequence -Gly-X-Y-, where X is often proline and Y is often hydroxyproline. After secretion in the extracellular compartment and removal of N- and C-propeptides, collagen triple helices aggregate into quarter-staggered fibrils. Newly synthesized collagen fibrils are soluble in salt solutions and dilute acid and have no tensile strength. During the formation of intermolecular cross-links, collagen fibrils become increasingly insoluble, more refractory to the action of enzymes and show a progressive increase in tensile strength. The cross-linking process is initiated by the enzyme(s) lysyl oxidase(s) (LOX), through the oxidation of specific lysine or hydroxylysine residues in the telopeptide regions. The resulting aldehydes undergo a series of reactions with adjacent reactive residues to give both inter- and intramolecular cross-links [52].

Elastin is the most abundant protein of the wall of the large arteries, which are subjected to an important pulsatile pressure generated by cardiac contraction [11,25,55]. However, elastin is also detectable in the resistance arteries — mainly in the internal and external elastic lamina — and in veins. Elastin represents 90% of the elastic fibers, the other constituents being microfibrillar glycoproteins such as fibrillins and microfibrillar-associated glycoproteins (MAGPs). The precursor of elastin, tropoelastin, is a highly hydrophobic protein (MW, 72 kDa), which is soluble in salt solutions like the collagen triple helix. In contrast to tropoelastin, elastin is an insoluble protein. This insolubility results from the cross-linking between lysine residues. Cross-linking of tropoelastin molecules begins with the oxidative deamination of some lysine residues by LOX(s), as previously described for collagen cross-linking. The spontaneous condensation reaction
between four lysine/allysine residues subsequently leads to the formation of the specific cross-links for elastin, desmosine and isodesmosine. Cross-links resulting from the condensation of two or three lysine/allysine residues are also detectable in elastin [52]. This cross-linking process confers to elastin its principal function — i.e. elasticity — essential in large arteries, which distend during systole and recoil during diastole. Another property of elastin has been recently demonstrated by the study of patients suffering from supra-valvular aortic stenosis and of knock-out mice for elastin: elastin controls, directly or indirectly, the proliferation and phenotype of smooth muscle cells [36,37,47].

Five human LOX isoenzymes have now been characterized; the contribution of each one to collagen and elastin cross-linking is unknown. The inactivation of the first described gene, LOX, leads to the death of homozygous mice at parturition or within the first few hours after birth [23,41]. Death is due to rupture of arterial aneurysms and diaphragm. Microscopic analysis of the aorta showed fragmented and disorganized elastic fibers whereas the number and diameter of collagen fibers appeared unchanged. The desmosine content of the aorta and lung was decreased by 60% and total body immature collagen cross-links (dihydroxy- and hydroxylsinosinonorleucine) were reduced by 40%. Thus, the lysyl oxidase isoform LOX appears to play a central role in the development and function of the cardiovascular system and diaphragm. The role of the four lysyl oxidase-like (LOXL) isoforms remains to be elucidated.

MAGPs and fibrillins are structural glycoproteins closely associated with elastin in elastic fibers [20,21,30]. Fibrillin-1 and -2, 350 kDa glycoproteins, are characterized by the multiple repeats of EGF-like domains, in which the three-cystine bridges stabilize the β-turns within the molecule. A recently discovered protein, fibulin-5, also plays a critical function during elastic fiber fibrogenesis as it acts as a bridge between elastic fibers and cells through its interactions with elastin on one side and integrins on the other side [48,64].

Several other structural glycoproteins play an essential role in the structure and function of the ECM in the arterial wall, including fibronectin, vitronectin, laminin, entactin/nidogen, tenascin and thrombospondin. These glycoproteins have a multidomain structure, potentially enabling simultaneous interactions between cells and other ECM components [8,32].

The glycosaminoglycans and PGs contained in the vascular wall are hyaluronan, the large aggregating PG versican, the small non-aggregating interstitial PGs biglycan, decorin and lumican and the cell-associated PGs syndecans, fibroglycan and glypican. PGs are proteins that have one or more attached glycosaminoglycan chains [24,49]. They contain distinct protein and carbohydrate domain structures, which interact with other ECM molecules. They participate in ECM assembly and confer specific properties to the tissues (hydration, filtration,...). PGs also regulate various cellular activities (proliferation, differentiation, adhesion, migration) and control cytokine biodisponibility and stability (FGF, TGF-β,...) [40].

2. The proteinases/inhibitors involved in vascular ECM remodeling

The metabolic turnover of mature collagen and elastic fibers in adult animals is relatively slow [57]. Although only small amounts of these proteins are degraded normally, increased degradation and fragmentation of collagen and elastin fibers are observed in vascular diseases. Collagenolytic and elastinolytic enzymes are found in a number of mammalian cells and tissues including polymorphonuclear neutrophils (PMN) and monocytes/macrophages. Collagenolytic enzymes are mainly matrix metalloproteinases (MMP). Elastinolytic enzymes are found in all the four classes of proteinases (serine, metallo-, aspartic and cysteine proteinases) and react with a broad spectrum of substrates [53,63]. All elastases studied to date have catalytic activity against protein and peptide substrates other than elastin.

MMPs and other elastolytic enzymes have been described extensively in previous reviews [7,12,14,18,61]. We will focus only on the serine and metalloproteinases, which participate in vascular ECM remodeling, i.e. proteinases synthesized by vascular cells themselves and those secreted by inflammatory cells (Table 2).

The two serine proteinases involved in vascular remodeling are SMC elastase [51] and leukocyte elastase, which is stored in the azurophil granules of PMN [6]. One inhibitor of

| Table 2 |
| Proteinases detectable in vascular and inflammatory cells |
| Proteinases produced by vascular cells | Proteinases produced by inflammatory cells | Inhibitors |
| MMPs: | MMPs: TIMPs, α2-macroglobulin |
| Collagenases: MMP-1 a | Collagenases: MMP-1 (MΦ), MMP-8 (PMN), MMP-13 (MΦ) |
| MMP-9 a | Gelatinases: MMP-2 (MΦ, lymphocytes), MMP-9 (MΦ, PMN) |
| “Elastases”: MMP-7 | “Elastases”: MMP-7 (MΦ), MMP-12 (MΦ) |
| Stromelysins: MMP-3 a | Stromelysins: MMP-3 (MΦ) |
| MT-MMPs: MMP-14 |
| Serine proteinases: | Serine proteases: α1-Antitrypsin, α2-macroglobulin, elafin |
| SMC elastase | Leukocyte elastase (PMN) |
| Cathepsin G (PMN) |
| Cysteine proteinases: | Cysteine proteases: Cathepsin S (MΦ) |
| Cathepsin K (MΦ) |

* Enzymes not expressed in basal conditions [18].
SMC elastase is elafin and leukocyte elastase is inhibited by α1-proteinase inhibitor, α2-macroglobulin and elafin.

MMPs play a fundamental role in the degradation of vascular ECM. MMPs include collagenases (MMP-1, expressed by endothelial cells, MMP-8, stored in specific granules of PMN), gelatinases (MMP-2, constitutively expressed by vascular cells; MMP-9, expressed in macrophages and PMN, inducible in vascular cells), “elastases” (MMP-7, expressed at a low level by the vascular wall, MMP-12, synthesized during the differentiation of monocytes into macrophages), stromelysins and membrane-type metalloproteinases (MT-MMPs).

The proteolytic activity of each MMP is tightly regulated at three levels: first, gene expression and protein secretion levels; second, activation of the inactive pro-enzyme, and, third, inhibition by the tissue inhibitors of matrix metalloproteinases (TIMPs) or other inhibitors (α2-macroglobulin). The activation of secreted pro-MMPs requires the disruption of the Cys–Zn$^{2+}$ interaction (cysteine switch) and the removal of the pro-peptide. Pro-MMPs can be activated in vitro by SH-reactive agents, mercurial compounds, SDS or chaotropic agents. In vivo, pro-MMPs are activated by tissue or plasma proteinases — plasmin, thrombin, other MMPs or MT-MMPs — and reactive oxygen species. The activation of MT-MMPs occurs intracellularly by furin. According to our experiments [4,15,19], the activation of MMPs and their inhibition by TIMPs are the main regulatory mechanisms of MMP activities in the vascular wall. For example, pro-MMP-2 is constitutively expressed in the normal arterial wall, in which no SMC migration or elastin degradation are detectable. The very well-controlled balance between active proteases and inhibitors is perturbed during pathological processes, particularly when PMNs or macrophages are present. However, through their capacity to synthesize enzyme inhibitors, smooth muscle cells have an extremely high capacity to respond to these increased enzyme levels.

3. ECM remodeling in arteries during aging

In humans, the intima-media thickness of the carotid increases two to threefold between 20 and 90 years of age [33]. This age-associated increase in the intima-media thickness is linked with both luminal dilation and increased wall stiffness. This has also been demonstrated in rats [43]. Using normotensive WAG/Rij rats which have a constant systolic blood pressure between 6 and 30 months of age, Michel et al. [43] demonstrated that the absolute elastin content of the aorta did not change with aging; however, the relative elastin content decreased as the collagen content showed a twofold increase in 30-month-old animals compared to 6-month-old animals. These changes in elastin and collagen result in a decrease in arterial distensibility and systemic compliance.

Aging was also associated with an increase in medial thickness, which was mainly due to smooth muscle cell hypertrophy [43]. However, the relative thickening of the intima is greater than the thickening of the media [38,43]. The sub-endothelial space in old rats is composed both of ECM (collagens, PGs, fibronectin,...) and smooth muscle cells. In parallel to these modifications of ECM composition, Lakatta et al. [34,38] described a threefold increase in MMP-2 protein content and a similar increase in the MMP-2 activity in the aorta of 30-month-old rats compared to 6-month-old rats. The active MMP-2 is mainly located in the thickened intima as shown by in situ zymography. This increased activation of the MMP-2zymogen in the intima of old rats is associated with an increase in MT1-MMP and plasminogen activators (PAs) without any increase in their respective inhibitors, TIMP-2 and PAs [62]. In these studies, TIMP-1, another inhibitor of active MMP-2, was not measured. Nevertheless, the detection of active MMP-2 in the thickened intima by in situ zymography revealed a local imbalance between MMP-2 and its inhibitors.

The modifications observed in old “normal” arteries i.e. intima-media thickening, dilation, increased stiffness and endothelial dysfunction predict a higher risk for developing atherosclerosis, hypertension, stroke and heart failure. This can explain the great age-dependent increase in vascular diseases [33]. However, the contribution of the various serine proteases and MMPs in these vascular diseases differs according to the relative number of inflammatory cells and mesenchymal cells and the localization of these two cell types.

4. ECM remodeling in arteries during hypertension

The ECM content (collagen + elastin) of arteries is increased in hypertensive patients and in genetic or experimentally induced hypertension in animals. The increase in collagen and elastin synthesis induced by the increased blood pressure has been carefully studied by Keeley et al. [27–29]. The collagen and elastin synthesis returns to basal levels as soon as the blood pressure stops rising. While the absolute aortic contents of elastin and collagen are increased in hypertensive animals, the elastin/collagen ratios are the same in normotensive and hypertensive animals.

These increased collagen and elastin contents in hypertensive animals may also result from reduced degradation. The MMP/TIMP balance has been studied in the rat model of hypertension induced by chronic blockade of NO production by Nω-nitro-L-arginine methyl ester [19]. Pro-MMP-9 was not detected in the aortic extracts of normotensive and hypertensive rats. Pro-MMP-2 activity, as well as TIMP-2 inhibitory capacity, were not significantly modified in the aortas of hypertensive rats. In contrast, TIMP-1 inhibitory capacity, measured by reverse zymography, and TIMP-1 mRNA levels were significantly increased in hypertensive rats. These data suggest that hypertension is associated with the overexpression of both pro-inflammatory molecules (interleukin-6, monocyte chemoattractant protein-1 and macrophage colony-stimulating factor) and resistance factors such as TIMPs. TIMP-1, by inhibiting MMP activity, limits inflam-
matory cell migration into the media and leads to a subsequent increase in ECM content.

Interestingly, it was recently demonstrated that serum concentration of free TIMP-1 is increased, while serum concentration of MMP-1 is diminished, in patients with essential hypertension. Furthermore, free TIMP-1 concentration was decreased, and free MMP-1 concentration was increased, in the sera of same patients after 1 year of treatment with an angiotensin-converting enzyme (ACE) inhibitor[35].

Taken together, these studies demonstrate that the synthesis of ECM is enhanced and its degradation reduced in the arteries of hypertensive individuals.

5. ECM remodeling in aneurysms

Human abdominal aortic aneurysms are characterized by progressive dilatation that may lead to the rupture of the aortic wall[44,45]. Mutations in genes encoding type III collagen and fibrillin-1 are responsible for several forms of inheritable aneurysmal-like disease with vascular rupture, such as Ehlers–Danlos type IV and Marfan’s syndrome[47]. However, the great majority of abdominal aneurysms develop as acquired degenerative lesions in the absence of known ECM genetic defects[22]. Associations between aneurysms and aging, atherosclerosis, male gender, as well as smoking and inflammatory reactions have been established. The ECM remodeling in aneurysmal aortas is characterized by the structural disorganization and disappearance of the elastic lamellae in association with an inflammatory, adventitial fibrosis. The inflammatory process is characterized by infiltration of the outer aorta by mononuclear phagocytes and lymphocytes. Another biological phenomenon observed during the occurrence of abdominal aortic aneurysms is the invasion of the media by capillaries; hence, proliferative endothelial cells are detectable in aneurysmal aortas. ECM degrading enzymes secreted by both invading and resident cell types are characteristic of all these processes. In fact, increased proteolytic activities have been described in aneurysmal aortas, including serine elastases, MMPs and PAs, these proteinases being directly or indirectly involved in aortic elastin degradation. The serine elastase involved in aortic aneurysms is neutrophil elastase. The main MMPs detected in aneurysmal aortas have elastolytic activities: MMP-2, constitutively expressed by vascular cells in normal aortas and MMP-9 and -12, both expressed in particular by tissue macrophages present in the outer aneurysmal aorta[10,59]. The quantities of TIMP-1 and -2 in aneurysmal tissues have been measured at the mRNA and protein levels, and are significantly increased in pathological tissues[58,59].

A thrombus is often detectable in the dilated aneurysmal abdominal aorta. Recently, we demonstrated that this intraluminal thrombus contains proteases, in particular MMPs and enzymes of the fibrinolytic system. These enzymes can be activated in the liquid phase sometimes present at the interface between the thrombus and the arterial wall and observable by computed tomography scan[16]. Both thrombus and arterial wall contain MMP-2 and -9 but the ratio MMP-9/MMP-2 is higher in the thrombus than in the arterial wall. The high content of MMP-9 in the thrombus is associated with PMNs trapped in the luminal part of the thrombus. This thrombus also contains plasminogen whereas its activators uPA and tPA are only detected in the arterial wall. Plasminogen could thus be activated at the interface between the thrombus and the arterial wall; this activation can lead to partial fibrinolysis of the thrombus and consequently to the apparition of the liquid interface and to activation of MMPs. These activated MMPs could further hydrolyze the remaining ECM of the arterial wall and contribute to the evolution of the aneurysmal aorta towards enlargement and rupture. Our findings support the previous demonstration that the gelatinase proteolytic activity, visualized using in situ zymography, was detected in the luminal portion of aneurysmal aortas[31]. Our data can also explain the increased MMP-9 level in the plasma of aneurysmal patients, MMP-9 being released from neutrophils during clot formation; this MMP-9 decreases to basal levels when the thrombus is efficiently excluded after endovascular graft placement[56]. In contrast to MMP-2 and -9, MMP-3 and -7 were only present in the aneurysmal arterial wall and not in the thrombus.

Finally, the well-described decrease in medial SMC density in aneurysmal aortas compared to normal or occlusive aortas may explain the lower production of MMP inhibitors relative to proteinases in aneurysmal aortas[39]. Indeed, the local seeding of SMC, whether they overexpress TIMP-1 or not, prevents elastin depletion, aneurysm formation and rupture in a rat model of aneurysm[1,3]. The blockade of MMP activation also prevents both early aneurysmal changes and the incidence of induced aneurysms in rat models of aortic and cerebral aneurysms[2,17].

6. ECM remodeling in the varicose veins

The wall thickness of the normal saphenous vein is regular and the media, constituting the major part of the venous wall, consists of three concentric muscle layers. On longitudinal sections, the inner and outer layers appear as thin, clustered longitudinal bundles. The large intermediate layer contains circular bundles of smooth muscle cells. The elastic network consists of a thin and continuous internal elastic lamina, regular longitudinal medial elastic fibers and large adventitial ribbons. Primary varicose veins are functionally characterized by venous back-flow and increased blood stasis in the upright position. Dilatation and tortuosity provide evidence for progressive venous wall remodeling, with disturbance of smooth muscle cell/ECM organization. In a previous historical study, we showed that affected areas are not uniformly distributed, some areas being hypertrophic, whereas others are atrophic or unaffected[5]. Numerous areas of intimal thickening were observed. In hypertrophic portions, medial smooth muscle cells were hypertrophic and showed marked alterations of smooth muscle α-actin, desmin and
vimentin staining suggesting modulation from contractile to proliferative and synthetic phenotype. Furthermore, the organization of the three concentric muscle layers was greatly disturbed: accumulation of ECM broke up the regular pattern of smooth muscle bundles. In contrast, in atrophic portions, both cellular and matrix components were reduced. The medial elastic network was highly disorganized and fragmented in both hypertrophic and atrophic portions.

When the proteolytic MMP/TIMP balance, which may participate in the remodeling of the venous wall, was investigated, a higher TIMP-1 level, a similar quantity of TIMP-2, and lower MMP-2 level and activity (based on soluble protein content) were found in varicose veins compared to control veins [4]. Consequently, the protein ratios TIMP-1/MMP-2 and TIMP-2/MMP-2 were 3.6- and 2.1-fold higher in varicose veins compared to control veins, respectively. Pro-MMP-9 was detected in variable amounts, both in control and varicose veins, but the mean activity was lower in varicose veins. The lower level of pro-MMP-2, constitutively expressed by smooth muscle cells and endothelial cells, probably reflects a lower production of pro-MMP-2 by cells of the varicose veins. In contrast, the increase in TIMP-1, detectable in both endothelial cells and smooth muscle cells, indicates a higher expression of the TIMP-1 gene by vascular cells. Indeed, depending on the cell type, TIMP-1 expression can be modulated by various cytokines (tumor necrosis factor-α, interleukin-1β, platelet-derived growth factor, transforming growth factor-β (TGF-β)) and soluble factors (angiotensin II, phorbol myristate acetate, retinoids). All these molecules have not been measured in varicose veins, but an increase in the total TGF-β1 concentration has been reported [5]. In turn, TGF-β1 down-regulates MMPs and up-regulates TIMP-1. This stimulatory effect of TGF-β1 on TIMP-1 expression is additive to the stimulation of collagen and elastin synthesis by TGF-β. Hypoxia is also a potential candidate as a modulator of TIMP-1 expression. This imbalance between MMP and TIMP production, in favor of an antiproteolytic activity, may explain, at least in part, the accumulation of ECM in varicose veins.

As plasmin activates some pro-MMPs and degrades some ECM molecules, the components of the fibrinolytic system, including tissue-type (tPA) and urokinase-type (uPA) plasminogen activators (PAs) and plasminogen activator inhibitors (PAIs) were also measured in vein extracts. Both uPA and tPA activities, as well as the amount of PAI, were lower in varicose veins and the ratio PAI/uPA + tPA was slightly lower in varicose veins than in control veins [4].

The pathogenic mechanisms leading to varicosis is not well known. Michiels et al. suggested the involvement of PMN adhesion to endothelial cells activated by hypoxia [46]. By measuring secreted proteins or solubilized membrane proteins from endothelial cells or leukocytes in blood samples in a control vein (brachial vein) and a varicose vein, before and after 30 min of blood stasis, we further demonstrated that, after their adhesion, neutrophils were activated and they released their granule content [26]. In the control vein, stasis did not induce significant modifications of endothelial markers (von Willebrand factor, thrombomodulin, soluble ICAM-1 (sICAM-1), soluble VCAM-1 (sVCAM-1), ACE, vascular endothelial growth factor (VEGF)) or leukocyte markers (lactoferrin, myeloperoxidase, interleukin-8, L-selectin, MMP-9). But, blood stasis induced in the varicose vein a significant increase in sICAM-1, sVCAM-1, ACE, L-selectin and MMP-9. The marked increase in plasma MMP-9 activity provides evidence for neutrophil activation and granule release in the varicose vein in response to postural blood stasis. And, detection in plasma of membrane proteins shed from the endothelium or leukocytes provides evidence of pericellular proteolysis.

7. Conclusion

In uninjured arteries and veins, some MMPs are constitutively expressed (Table 2). But through the control of their activation and their inhibition by inhibitors synthesized by the vascular cells themselves (TIMPs), these proteases have a very low activity.

During aging and the occurrence of vascular pathologies, this balance between proteases and their inhibitors is temporally destroyed through the induction of MMP gene expression in vascular cells or the secretion of enzymes by inflammatory cells. If the protease levels exceed only for a short time the inhibitor level, this may then be overcompensated and the normal healing and repair process would lead to the deposition of newly synthesized ECM components and the arrest/inhibition of SMC proliferation. However, during the long-term evolution of the principal vascular pathologies (atherosclerosis, aneurysm) a minimal imbalance between degradation and synthesis of ECM components could lead either to stenosis or to dilatation of the vessel wall. Furthermore, depending on the extent of the degradation and synthesis of each ECM component, the relative level of each component, and especially the elastin/collagen ratio, may be modified. In addition, the three-dimensional organization of this newly synthesized ECM, and consequently its functional properties, are never optimal as are those of ECM synthesized during development.

In conclusion, care should be taken to maintain the vascular ECM reserve and any therapeutic manipulation of the protease/inhibitor balance must be perfectly controlled, because an accumulation of abnormal ECM may have unforeseen adverse effects.

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