REVIEW

Preventing restenosis after angioplasty: a multistage approach

Ramin ZARGHAM
Division of Experimental Medicine, McGill University, 110 Pine Avenue, Montreal, Quebec, Canada H2W 1R7

ABSTRACT

Arterial reconstruction procedures, including balloon angioplasty, stenting and coronary artery bypass, are used to restore blood flow in atherosclerotic arteries. Restenosis of these arteries has remained a major limitation of the application of these procedures, especially in the case of balloon angioplasty. Post-angioplasty restenosis results from two major processes: neointimal formation and constrictive remodelling. Neointimal formation is initiated by arterial injury with a resultant loss of contractile phenotype in tunica media, leading to VSMC [vascular SM (smooth muscle) cell] migration from the tunica media to the intima. Migrated VSMCs contribute to the intimal thickening by the excessive synthesis of ECM (extracellular matrix) and proliferation. However, increased neointimal mass is not solely responsible for luminal narrowing. Inward constrictive remodelling is also considered as a major cause of delayed failure of angioplasty. At later stages after angioplasty, the increase in contractile forces leads to lumen narrowing. Recent studies show that SM contractile proteins are re-expressed in the neointima, concomitant with late lumen loss. Therefore one important question is whether the restoration of contractile phenotype, which can suppress VSMC migration, is favourable or detrimental. In this review, the importance of viewing restenosis as a multistage process is discussed. Different stages of restenosis occur in a sequential manner and are related to each other, but in each stage a different strategy should be taken into consideration to reduce restenosis. Defining the role of each process not only reshapes the current concept, but also helps us to target restenosis with more efficacy.

INTRODUCTION

Atherosclerotic stenosis and its ischaemic complications necessitate arterial reconstruction. Current therapeutic strategies to restore blood flow in stenotic coronary arteries include percutaneous transluminal angioplasty, intracoronary stents and coronary artery bypass surgery. Regardless of the method employed, the incidence of restenosis is high: up to 40 % within 6 months after percutaneous transluminal angioplasty [1] and up to 20 % after bypass surgery [2]. Moreover, the trend towards primary stenting has not significantly improved the patency rate [3]. Therefore restenosis remains the Achilles' heel of coronary intervention, requiring continuous advances in our understanding of its mechanism and new innovative solutions.

Restenosis is arbitrarily defined as a greater than 50 % narrowing of vessel diameter compared with a reference artery. Restenosis arises from two major processes: neointimal formation and constrictive remodelling. In the acute phase after injury, neointimal formation is initiated by arterial injury, which evokes loss of the contractile phenotype in the tunica media. Loss of the VSMC contractile phenotype facilitates its migration from the tunica media towards the tunica intima. Formation of the neointima

Key words: angioplasty, cell migration, neointima, remodelling, restenosis, vascular smooth muscle cell.

Abbreviations: EC, endothelial cell; ECM, extracellular matrix; MAL, megakaryocytic acute leukaemia; MLC, myosin light chain; PDGF, platelet-derived growth factor; ROCK, Rho kinase; SM, smooth muscle; SMC, SM cell; SRF, serum-response factor; TGF-β, transforming growth factor-β; VSMC, vascular SMC.

Correspondence: Dr Ramin Zargham (email r_zargham@hotmail.com).
is followed by the inward remodelling of the vascular architecture, with variable degrees of arterial narrowing in the chronic phase [4–6]. A solution to restenosis must lie in a detailed understanding of the events causing neointimal thickening and vascular remodelling. Once the contribution of each is understood, interventions can be strategically planned, demonstrated in animal models and successfully translated into human therapy.

**MECHANISM OF NEOINTIMAL FORMATION**

Neointimal formation after injury entails the generation of new tissue at a location which had none previously. During arterial catheterization, the endothelial layer is removed by balloon dilation, resulting in the loss of this important antithrombogenic layer. Moreover, endothelial denudation and medial tearing result in the exposure of circulating blood cells to the subendothelial matrix. The exposed subendothelial matrix contains numerous platelet-activating factors, including thrombin, thromboxane, platelet-activating factor and collagen. Thrombin generation elicits further platelet activation [7]. The exposed deeper surfaces are thrombogenic, and immediate thrombus formation occurs [8]. These thrombi contain chemotactic and mitogenic factors and may function as a matrix for the migration and proliferation of VSMCs. In fact, the release of cytokines and growth factors from activated platelets and macrophages culminates in the phenotype modulation of VSMCs and subsequent migratory activity. Transformed VSMCs migrate toward the source of stimulation and produce a neointima. Later on, the neointima is developed by excessive matrix synthesis and the accumulation of different types of cells, including migrated VSMCs, circulating progenitor cells [9] and myofibroblasts originating from the adventitia [10].

VSMCs are the most prominent cell type in the media of mammalian arteries. They are responsible not only for vascular tone and integrity, but also for post-injury events. It is pertinent to explain further the biology of VSMCs. What are these cells? What is the phenotype of mature VSMCs?

**VSMC-DIFFERENTIATED PHENOTYPE**

There are three classes of muscle: skeletal muscle, cardiac muscle and SM. SMs surround the internal organs, such as the large and small intestines, the uterus, the respiratory tract and blood vessels. Muscle cells have evolved to perform a highly specialized function: contraction. These cells contract either spontaneously or in response to agonists.

Cellular differentiation is simply the process by which multipotential cells in the developing organism acquire cell-specific characteristics that distinguish them from other cell types. Therefore, as the primary function of VSMCs is contraction, during the process of development VSMCs are highly differentiated for contractile function. This differentiated state of VSMCs is also called the contractile phenotype. VSMCs within the adult vasculature express a unique repertoire of contractile proteins, ion channels and signalling molecules, all of which are required for contractile function [11]. However, the contractile phenotype is not simply due to the expression of contractile proteins, but also to the assembly of actin fibres into organized stress fibres, and the development of focal complexes into mature focal adhesions [12,13].

Smoothelin, SM α-actin, SM myosin heavy chain, calponin, α1 integrin, α8 integrin and α-tropomyosin [14–16] are all VSMC contractile proteins.

Analysis of the regulatory regions of many of these genes has revealed the presence of a 10-bp cis-element called the CArG box [17,18]. The CArG element binds the highly regulated and interactive transcription factor SRF (serum-response factor) [18], which is responsible for the expression of SMC-differentiation genes. SRF is bound to CArG-containing regions in multiple SMC marker genes within intact chromatin [19]. Some other transcription factors which play an important role in SMC gene expression are Sp1 (stimulated protein 1), MEF2 (myocyte enhancer factor 2), AP2 (activator protein 2), Mbox and YY1 (Ying-Yang 1) [17,20]. Moreover, myocardin, a protein that is highly expressed in the heart, acts as a potent transcriptional co-activator for SRF [21]. Myocardin directly binds to SRF, transactivates SM22α and ANF (atrial natriuretic factor) promoter-reporter genes in a CArG-dependent manner. Myocardin is also expressed in the aorta [22] and is thought to be a master regulator of SM gene expression [23]. Activation of SRF also depends on the binding of another co-activating factor, MAL (megakaryocytic acute leukaemia), one of the myocardin-related transcription factors [24]. Previous reports have documented that increased SM α-actin availability inside the cytoplasm, due to actin stress fibre breakdown into its building blocks, sequesters this cofactor in the cytoplasm [24]; therefore SRF could not activate the expression of SMC genes. In contrast, decreased availability of G-actin in the cytoplasm, associated with actin polymerization, results in the nuclear accumulation of MAL, where it co-activates the transcription of genes containing SRF sites in their promoters [24]. In this regard, alterations in actin dynamics are necessary and sufficient for SRF activation [25], and the expression of the SM-specific gene depends on the existence of stress fibres [25].

Actin exists as a globular monomer called G-actin and as a filamentous polymer called F-actin, which is a linear chain of G-actin subunits. The ability of G-actin to polymerize into F-actin and of F-actin to depolymerize into G-actin is an important property of actin. In mature and differentiated VSMCs, actin fibres are formed as stress fibres, plasma membrane-linked, long cytoskeletal...
RhoA SIGNALLING AND THE VSMC PHENOTYPE

Although signal transduction in the regulation of the VSMC phenotype machinery is still obscure, RhoA is thought to play a central role [25,26]. The Rho family of small G-proteins consists of three major classes, including Rho, Rac and CDC-42. RhoA, a member of the Rho class, acts as a molecular switch that cycles between an active GTP-bound RhoA and an inactive GDP-bound form [27]. RhoA has been shown to partially translocate from the soluble (cytosolic) to the particulate fraction (membrane) upon activation [28].

ROCKs (Rho kinases) were the first and are the best-characterized RhoA effectors. RhoA activation induces actin polymerization through two effector pathways: the ROCK/LIM kinase/cofilin pathway, which stabilizes F-actin [29], and the mDia1 pathway, which promotes its assembly [30]. RhoA is recognized as a major regulator of cell contraction through ROCK. The major regulatory mechanism of SM contraction is the phosphorylation/dephosphorylation of MLC (myosin light chain), which is regulated by ROCK. Interestingly, even in the absence of vasoconstrictors, Rho/ROCK pathway is active to maintain basal tone and SMC contractile ability [31]. This is in line with the phenotype-regulatory role of RhoA. RhoA appears to integrate multiple signals that regulate the SMC contractile state and, through the regulation of SRF-dependent transcription and actin polymerization [32], its activity is critical for controlling VSMC differentiation [25], assembly of actin stress fibres and focal adhesions [33,34].

VSMC PHENOTYPE MODULATION IN THE TUNICA MEDIA

VSMCs are highly plastic cells. Unlike skeletal and cardiac muscle cells that are terminally differentiated, VSMCs can undergo phenotypic transition in response to environmental stimuli, and that phenotypic transition is reversible.

Within 1 week after removal of the endothelium, VSMCs in the innermost part of the media take on a synthetic phenotype, as revealed by the down-regulation of VSMC-differentiated phenotype markers and the up-regulation of de-differentiated phenotype (Table 1). Moreover, in phenotype-modulated VSMCs, there is a loss of myofilaments and the outgrowth of an extensive endoplasmic reticulum with a large Golgi complex [35].

This new phenotype facilitates VSMC migratory activity. Migration of VSMCs from the tunica media towards the intima is thought to be a key event in neointimal formation.

In the contractile state, tensile forces exerted at the connecting point between the cell–ECM result in the development of focal adhesions from focal complexes and the assembly of the parallel actin stress fibres [13,26]. The consequence of this feature of VSMC–ECM interaction is excessive adhesiveness and contractility accompanied by reduced migratory activity [36]. These contractile forces inhibit the release of adhesions at the trailing edge, which are required to translocate the cell body [36]. Therefore, in this phenotype, cells cannot reach their maximal motility, since turnover of focal adhesion is needed for locomotion [37]. PDGF (platelet-derived growth factor)-BB released from the injury site is one of the most potent suppressors of contractile gene expression [38]. PDGF-BB can induce the disassembly of stress fibres and is a

<table>
<thead>
<tr>
<th>Table 1 Phenotype-dependent markers of SMCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Markers which are up-regulated in the contractile phenotype are down-regulated in the non-contractile phenotype and vice versa.</td>
</tr>
<tr>
<td>SM phenotype-dependent markers</td>
</tr>
<tr>
<td>Smoothelin</td>
</tr>
<tr>
<td>SM MLC1 and 2</td>
</tr>
<tr>
<td>αl Integrin</td>
</tr>
<tr>
<td>α5 Integrin</td>
</tr>
<tr>
<td>α7 Integrin</td>
</tr>
<tr>
<td>Calponin</td>
</tr>
<tr>
<td>SM α-cortyponysin</td>
</tr>
<tr>
<td>h-Caldesmon</td>
</tr>
<tr>
<td>SM α-actin</td>
</tr>
<tr>
<td>Myocardin</td>
</tr>
<tr>
<td>Fibroblast-like tropomyosin</td>
</tr>
<tr>
<td>Kruppel-like factor 4</td>
</tr>
<tr>
<td>α5 Integrin</td>
</tr>
<tr>
<td>α2 Integrin</td>
</tr>
<tr>
<td>αβ Integrin</td>
</tr>
<tr>
<td>SFemb</td>
</tr>
<tr>
<td>t-Caldesmon</td>
</tr>
</tbody>
</table>
potent chemoattractant [39]. Therefore, in contrast with non-motile cells which have organized long stress fibres traversing the cell longitudinally, actin fibres in motile cells disintegrate into short bundles [26,39]. Disassembly of stress fibres during phenotype modulation leads to the rearrangement of actin filaments in the form of veil-like structures at the cell margins. After phenotype modulation and VSMC alteration to a polarized asymmetric shape, the cells are now ready to migrate toward the tunica intima, the source of chemoattractants.

After neointima generation, the neointimal mass becomes thickened and developed.

**NEOINTIMAL THICKENING**

In the neointima, migrated VSMCs begin to divide and secrete ECM components, so that a thick neointima forms within 1 week [35]. On the other hand, evidence suggests that SM-like cells within an injured blood vessel may also be derived from various other sources, including adventitial fibroblasts [40] and circulating ‘stem’ cells [41]. The first line of evidence is that circulating bone-marrow-derived SMC progenitor cells play a major role in vascular injury repair [42,43]. Circulating bone-marrow-derived cells are recruited on the luminal side of injured arteries. These immature cells are initially negative for markers of SMCs and ECs (endothelial cells). Therefore it is possible that such immature cells may be differentiated to SMCs in response to mechanical and humoral stimuli. In support of this hypothesis, it has been shown that blood cells contain progenitors that have the potential to differentiate into either ECs or SMCs [9]. Adventitial myofibroblasts are another cell type thought to be involved in neointimal thickening. It has been reported that adventitial fibroblasts migrate to the neointima [10]. These adventitial cells are SM-α-actin-positive and are thus presumed to be adventitial myofibroblasts. Therefore it is likely that the neointima has a heterogeneous cell population.

After neointimal formation, self-rearrangement of the neointima occurs as an adaptation to the new condition. This is called vascular remodelling.

**VASCULAR REMODELLING**

Vascular remodelling leads to either vessel enlargement (positive remodelling) or shrinkage (negative remodelling) [44]. It is thought that, in stenotic arteries, increased shear stress at the lesion site can initiate compensatory enlargement [45]. After balloon angioplasty, dissection extending into the intima and media may weaken segments of the arterial wall, thereby allowing enlargement in response to the aforementioned increased shear stress as intimal formation occurs. Moreover, the inflammatory response to balloon injury could initiate the remodelling process through the release of proteolytic enzymes.

Arterial remodelling may be precipitated during this process of pre-existing matrix degradation and new matrix component deposition. Therefore, in the early stages, the neointima respects the lumen by expansive remodelling; however, later on, remodelling becomes constrictive, resulting in lumen narrowing. In an analogy with wound healing in an injured artery concomitant with collagen deposition, the phenomenon of wound contracture occurs. The end result of wound contraction is the reduced size of granulation tissue volume by the reorganization of collagen fibrils [46]. Although expansive (outward) remodelling favours keeping the lumen open and restoring blood flow, constrictive (inward) remodelling causes narrowing of the lumen due to the increased contractile properties and shrinkage of the artery’s circumference [4,47]. Compensatory enlargement (positive remodelling) is associated with intimal growth, and vessel shrinkage (negative remodelling) is not directly linked with any change in intimal hyperplasia [48].

Negative or constrictive remodelling still plays an important role in the pathogenesis of coronary restenosis, even in the era of interventional stenting (e.g. arterial narrowing occurs proximally and distally to a stented segment) [49]. The assumption that an increase in neointimal mass is solely responsible for luminal narrowing has been challenged [50], and inward arterial remodelling has been identified as one of the prominent causes of delayed failure of angioplasty [51]. Therefore the complex mechanisms of lumen loss suggest that the pathophysiology of restenosis may be different in the early and late phases after vascular injury.

Although many studies have proposed that constrictive remodelling is responsible for late lumen loss and plays a major role in the failure of angioplasty, the mechanism of constrictive remodelling is still unclear.

**MECHANISM OF CONSTRICTIVE REMODELLING**

It seems likely that in the neointima there is a re-expression or just up-regulation of contractile markers [52]. We have shown previously [53] that, at 7 days after balloon injury in the rat carotid artery, there is a down-regulation of α8β1 integrin concomitant with the loss of contractile phenotype in the tunica media. On the other hand, we have reported recently [54] the up-regulation of SM α-actin and α8β1 integrin as contractile markers in the neointima at 21 days after balloon injury of rat carotid arteries (Figure 1). This up-regulation was concomitant with late lumen loss. Moreover, α8β1 integrin and SM α-actin were localized at the medial side of the neointima where there was an intense expression of collagen and fibronectin, two hallmarks of active constrictive remodelling [54]. At 28 days, when there was evidence of arterial shrinkage (reduced size of the neointima as well
Preventing restenosis after angioplasty: a multistage approach

Figure 1  Stenosis after balloon injury

(A) Schematic image of neointima formation and constrictive remodelling. The red background is the extracellular matrix. After vascular injury there is modulation of VSMC phenotype (represented here by the shape change from spindle-like to polygonal). L, lumen. Arrows point to the internal elastic lamina. (B) Photomicrographs showing haematoxylin/eosin-stained sections. After balloon injury there is an increasing neointimal formation at 7, 14, 21, and 28 days compared with sham-operated controls (Ctrl). At 21 days and after, lumen narrowing and reduced artery size are observed. 1, 2, and 3 on each image correspond to the adventitia, media and neointima respectively. (C) Photomicrographs showing the immunohistochemical staining for SMα-actin in rat carotid arteries after balloon injury. SMα-actin is down-regulated in the tunica media and, at 21 day, is up-regulated in the intima. Arrows indicate the internal elastic lamina. Figures 1(B) and 1(C) are adapted with permission from Zargham R. Pepin, J. and Thibault, G., (2007) α8β1 Integrin is up-regulated in the neointima concomitant with late luminal loss after balloon injury, Cardiovasc. Pathol. 16, 212–220. © (2007) Elsevier.

as arterial circumference), α8 integrin became more up-regulated and spread throughout the complete neointima. The up-regulation of α8 integrin, which is required for the maintenance [55] and also restoration [56] of the VSMC contractile phenotype, indicates that the restoration of contractile phenotype did occur. However, it does not show which cell types are expressing α8 integrin, as the cellular composition of the neointima is unclear and α8 integrin up-regulation has been documented in myofibroblasts derived from various tissues [7,30]. Therefore it is still unknown which cell types infiltrate the neointima. The possibility exists that migrated VSMCs, after reaching the neointima, may switch back to the contractile phenotype [27]. On the other hand, there is evidence of the presence of myofibroblasts in the neointima [28], which can contribute to constrictive remodelling [29]. In recent years, studies showing the presence of progenitor cells differentiated to SMCs [31] has also added to the complexity. Therefore one of the very critical studies suggested is an investigation to distinguish the cell types infiltrating the neointima.

Whether the neointimal cells are immigrated VSMCs which switch back to the contractile state or other cell types with myofibroblastic features, the consequence of increased contractile properties is late luminal narrowing, which raises the question of how up-regulation of contractile markers happens. It is known that the expression of collagen, fibronectin and SM α-actin or generally constrictive remodelling is regulated by TGF-β (transforming growth factor-β) [57], which plays a crucial role in late luminal loss. Increased neointimal expression of TGF-β, especially 14 days after injury in rat carotid arteries, is concomitant with SMα-actin up-regulation and inward remodelling [58]. In our studies, TGF-β stimulation of both fibroblasts and VSMCs populated in three-dimensional collagen matrices resulted in α8 integrin up-regulation associated with the increased expression of fibronectin [54]. This finding suggests that, in the conditions of increased TGF-β release, such as in the case of neointimal constrictive remodelling, there is an increase in the contractile proteins. Although it is speculative, it appears that the disturbed blood flow after neointimal growth may lead to the increase in cytokine release and TGF-β at the later stages, which can induce contractile phenotype by the excessive synthesis of collagen and fibronectin, followed by the activation of their integrin receptors such as α1β1 and α8β1. Whether, in this context, up-regulation of these integrins can induce signalling
resulting in the contractile phenotype and/or the restoration of the contractile characteristics of de-differentiated VSMCs is not known. In spite of the ambiguous cellular content of neointima, our studies [56] have shown that overexpression of α8 integrin in both fibroblasts and de-differentiated VSMCs can induce contractile characteristics, which were inhibited by RhoA inhibitors.

It has been reported that RhoA plays an important role in constrictive remodelling and its inhibition can reduce restenosis by blockade of constrictive remodelling [59]. Local delivery of Y-27632 (an inhibitor of ROCK) suppressed constrictive remodelling after coronary balloon angioplasty in pigs [59]. It is noteworthy to add that α8β1 integrin is required for the membrane localization of RhoA [60].

**LOSS OR RESTORATION OF CONTRACTILE PHENOTYPE: WHICH SHOULD BE TARGETED IN RESTENOSIS?**

In the early stages after vascular injury, loss of contractile phenotype in the tunica media leads to cell migration towards the intima, contributing to the genesis of the neointima. As mentioned above, however, restenosis can be viewed not merely as neointimal formation in response to balloon injury, but also as arterial remodelling in response to balloon injury and neointimal formation [61]. Although it has been documented that constrictive remodelling is more responsible than intimal hyperplasia for the luminal narrowing that occurs [48], it seems that both processes are playing important roles, but at different stages.

Our recent study has shown that at 7 and 14 days after balloon injury there is no lumen loss, in spite of neointima formation [54]. However, after 21 days post-angioplasty, the neointima stops growing when cells have already gained contractile properties, e.g. α8 integrin and SM α-actin up-regulation. Furthermore, loss of the contractile phenotype early after balloon injury and re-expression of contractile markers later on emphasize the biphasic nature of restenosis pathology. This finding is in line with a clinical trial which showed that lumen loss is a biphasic process involving early intimal thickening and late constrictive remodelling [47]. This expanded paradigm of arterial remodelling and neointimal formation may account, in part, for the lack of success in clinical trials to date. Therapy directed at both arterial remodelling and neointimal formation may be required to control restenosis after coronary interventions. When a biphasic nature is considered in restenosis after balloon injury, its treatment would require biphasic therapy. Effective interventions to ameliorate neointimal formation should be started early after balloon injury, whereas interventions to reduce constrictive remodelling should be started after the appearance of the contractile phenotype. However, different strategies may be applied. Early after balloon injury, the maintenance of the contractile phenotype, which inhibits VSMC migration, appears to be favourable; however, in the second phase of treatment, during constrictive remodelling, targeting the contractile proteins might reduce luminal narrowing.

**CONCLUSIONS**

Many controversies in vascular biology may be due to the multistage and complex nature of restenosis. On the other hand, several studies which claim to have found a solution to the problem have failed to reduce restenosis. One of the reasons for this failure is probably a simplistic approach to the complex pathology of restenosis, which can be called ‘cross-sectional viewing’. This kind of viewing focuses on only one part of the process, regardless of what is happening in another stage. For instance, several studies have introduced αvβ3 integrin as a novel target in restenosis therapy because of the significant reduction of neointimal formation after αvβ3 integrin blockade. However, over a prolonged course of time, αvβ3 blockade leads to constrictive remodelling and restenosis [62].

One of the methods to test the multistage approach is using drug-coated balloons to block PDGF-BB, a potent repressor of contractile phenotype. This method should be combined with a method to repress contractile phenotype in the late stage of restenosis. For instance, using gel matrices around the balloon-injured artery releasing siRNA (short interfering RNA) targeting a master regulator of contractile phenotype.

Any strategy to reduce restenosis should, first, consider restenosis as a multistage process, which requires complex and sometimes paradoxical approaches. Secondly, the pathophysiology of restenosis is different in the early and late phases after balloon injury. The mechanism of neointimal formation with regard to the phenotypic transition is contrary to constrictive remodelling, so it is important to know when to address neointimal formation and when to address constrictive remodelling. Treatment timing is very important. Beyond the application of agents which can retain the contractile phenotype early after balloon injury, drugs reducing the contractile phenotype later on may be of considerable interest. Thirdly, although RhoA activity is a major signalling pathway for the maintenance of the contractile phenotype and therefore could be used to reduce neointimal formation, recent studies [59] have shown that its blockade can reduce constrictive remodelling and restenosis. This finding emphasizes that if we turn off the contractile phenotype pathway at the right time, we could even reduce restenosis. Finally, distinguishing the cellular composition of the neointima is of fundamental importance. We need to understand the mechanisms underlying restenosis better.
as well as targeting cell-specific genes involved in this process.

Taken together, future investigations, both in animal and human models, are urgently needed to clarify the observed vascular reactions. Despite therapeutic advances, the challenge for the future is to understand the mechanisms involved better, as this is the only way to optimize effective treatment and to prevent restenosis.

ACKNOWLEDGMENTS

I sincerely appreciate Ms Catherine Boyd for the grammatical corrections and comments on English writing prior to submission.

REFERENCES


264 R. Zargham


Received 3 July 2007/1 August 2007; accepted 8 August 2007

Published on the Internet 15 January 2008, doi:10.1042/CS20070228