

ALX-chemy: adding spice to the inflammatory soup of atherosclerosis

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This editorial refers to 'The role of the FPR2/ALX receptor in atherosclerosis development and plaque stability' by M.H. Petri *et al.*, pp. 65–74, this issue.

1. Introduction

Nature often presents complexities, for example the formyl peptide receptor (FPR) 2/ALX that curiously can mediate either pro-inflammatory or pro-resolution of inflammation responses depending on the ligand it encounters. Formyl peptides originate from bacteria. The formyl peptide receptor may not only respond to products derived from pathogenic organisms but also from commensals in the intestinal and other endogenous microbiomes, whose contribution to atherosclerosis has garnered considerable recent interest.

The ALX designation derives from the response of this family of receptors to lipoxin A₄. This receptor binds other ligands relevant to atherosclerosis, including serum amyloid A (SAA). Given this variety of agonists, the role of FPR2/ALX in atherogenesis has proved confusing. The study by Petri *et al.* provides a comprehensive re-evaluation of the role of FPR2/ALX in atherosclerosis.¹ In a thorough series of investigations, these authors have established the presence of this receptor in specimens of human atherosclerosis, documenting its expression presence on inflammatory cells and intrinsic vascular wall cells in plaques but not in normal arteries. The messenger RNA encoding FPR2/ALX in the retrieved carotid endarterectomy specimens correlated with symptomatic cerebral ischaemia. Joined with these observations on human plaques, the authors showed delayed atherosclerosis and fewer inflammatory cells in LDL receptor-deficient mice with global lack of FPR2. Bone marrow chimera experiments suggested that bone marrow cell-derived FPR2/ALX participated in atherogenesis and inflammation. The compound mutant mice also showed a defect in collagen metabolism that conferred characteristics associated with a propensity to rupture in human plaques. Thus, the duality of FPR2/ALX not only depends on its ligands but also on the cell types that bear it. Its expression on inflammatory leucocytes appears to promote atherogenesis but its presence on smooth muscle cells associates with plaques that may prove less likely to rupture. This study derives strength from the

combination of observations on human specimens with mechanistic studies conducted *in vitro* and in mice.

2. The 'knock-out' conundrum in experimental atherosclerosis

Experimental studies in mice have implicated dozens, perhaps hundreds, of mediators in atherosclerosis using various loss- and gain-of-function approaches. Most of such studies use the now well-established compound mutant approach, as illustrated in the work of Petri *et al.* The aggregate catalogue of results produced by this enterprise presents a problem for the field. Most of the published reports describe a 20–60% reduction in murine atherosclerosis by interrupting a particular signalling mediator. Granted, a publication bias likely leads to underreporting of negative studies and the highly contrived mouse preparations that we use may not reflect the human disease that generally smoulders for decades. Compensatory mechanisms at play during development may distort the consequences of germline modifications. Moreover, few of the multiplicity of mediators inculcated in atherogenesis by mouse studies have emerged as causal or associated in contemporary genetic studies (GWAS). Yet taking the experimental literature at face value, how can atherosclerosis involve causally so many mediators that each seem to account for a substantial proportion of disease? The experimental literature suggests that atheroma should melt away by attacking just a handful of targets validated in mouse studies, such as in the Petri report. How can one reconcile this conundrum?

Thinking linearly, various mediators may fall into a common pathogenic pathway, such that they participate sequentially at different stages on the same road to disease (Figure 1). Several such linear, orthogonal pathways may converge on atherogenesis. For example, lipids, inflammation, oxidation, thrombosis, extracellular matrix metabolism, and the reader's own favourite mechanism not included in this list, may comprise linear spokes leading to the core disease process.

Alternatively, taking cues from the current ascendancy of systems biology, this conundrum might cause us to reconsider our traditional linear thinking. Mediators of different pathogenic nodes may interact in subtle ways and modulate each other's function positively or

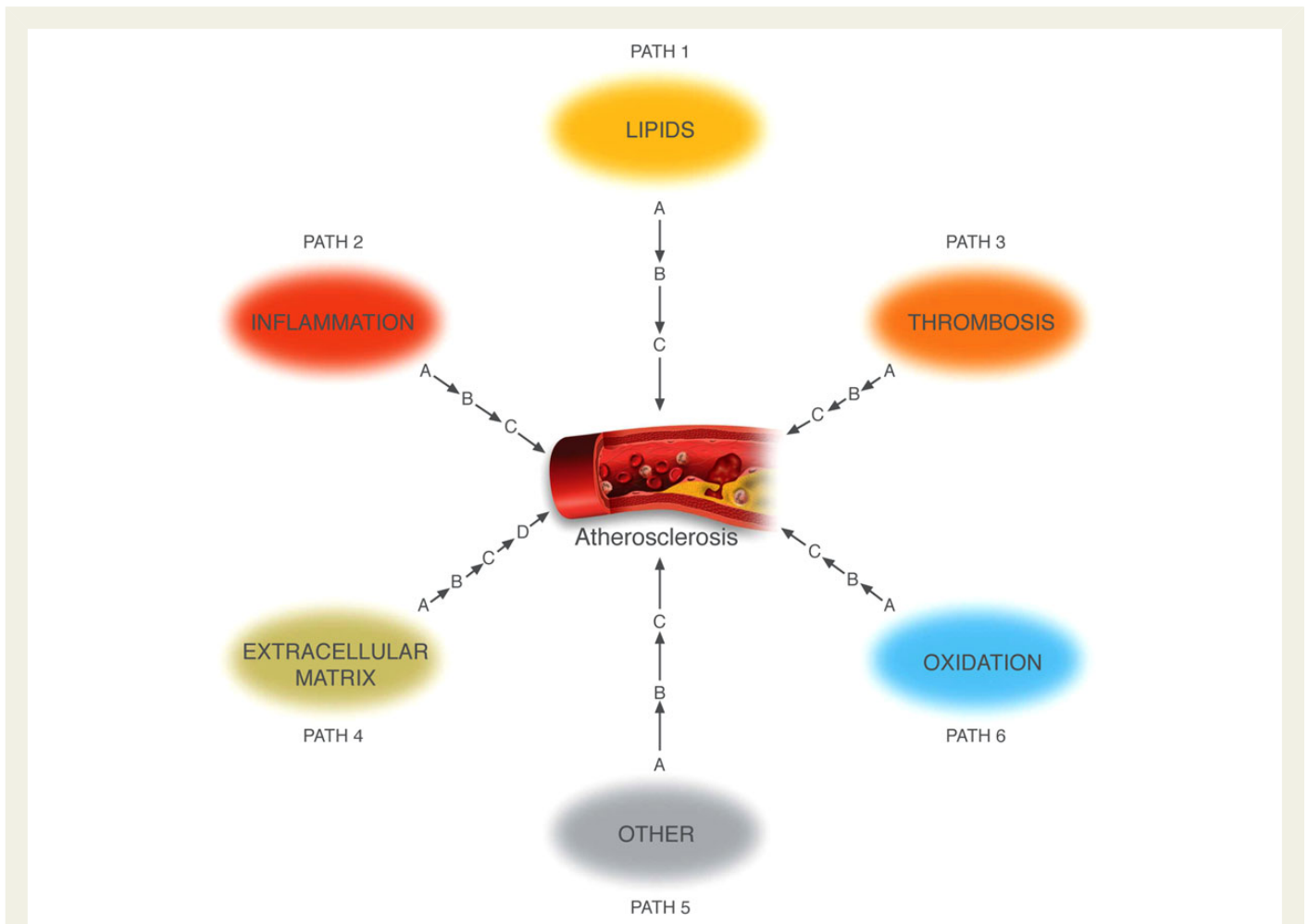


Figure 1 Can we reconcile the ‘knock-out conundrum’ in experimental atherosclerosis with a ‘linear’ concept of disease pathogenesis? Traditional linear thinking about biological mechanisms involves the contribution of single mediators (represented by the letters connected by arrows) operating in a succession of steps in an often hierarchical pathogenic path. Several such orthogonal linear pathways may converge to promote disease pathogenesis. Merging this traditional concept with the results of single-gene inactivation experiments suggests that attacking just a few targets should eradicate atherosclerosis, a *reductio ad absurdum*.

negatively (Figure 2). During the evolution of the human disease, a process that plays out over decades rather than weeks as in the mouse, such modulation and crosstalk might mitigate the consequences of loss of a single mediator as mimicked by a murine ‘knock-out’ experiment. Homeostatic and host defense mechanisms may constantly re-adjust and compensate for derangements, perhaps on a moment-to-moment basis, in a manner not reflected in the typical monoptic reductionist approach implicit in most of our mouse ‘knock-out’ studies. The bimodal responses of ligating FPR2/ALX on atherogenic mechanisms illustrate an example of such complexity.

3. Actionability of findings from experimental atherosclerosis studies

The body of data that has accumulated from contemporary studies on atherogenesis in mice has doubtless spurred greater understanding of the molecular and cellular bases of this disease. Yet to translate to treatment of the human disease, appropriate tools must exist to modulate the pathway *in vivo*. While receptors are attractive ‘druggable targets’, their

inhibition may entrain unwanted effects. Many of the mechanisms implicated in atherogenesis participate fundamentally in host defenses such that their manipulation may have undesired effects. By some phylogenetic fluke, humans generally have much higher LDL concentrations than a species requires for success. Hence, we have succeeded in driving down this causal risk factor for human atherosclerosis to subterranean levels seemingly without paying a high price in unwanted actions.

Other popular pathways may unfortunately not allow such latitude. We have reached a point in anti-coagulant and anti-thrombotic therapy where increases in bleeding counterbalance the beneficial effect in preventing cardiovascular events. Likewise, inflammation and reactive oxygen species help protect us from microbial pathogens. Immune pathways participate in tumour surveillance, raising the spectre that their inhibition to treat a chronic disease such as atherosclerosis might promote oncogenesis. Thus, to harness mediators identified in mouse ‘knock-out’ experiments therapeutically, we must not only find the practical tool to target them *in vivo*, but also find a ‘sweet spot’ where we interfere with their pathogenic actions without impairing host defenses, tumour surveillance, or other important functions.

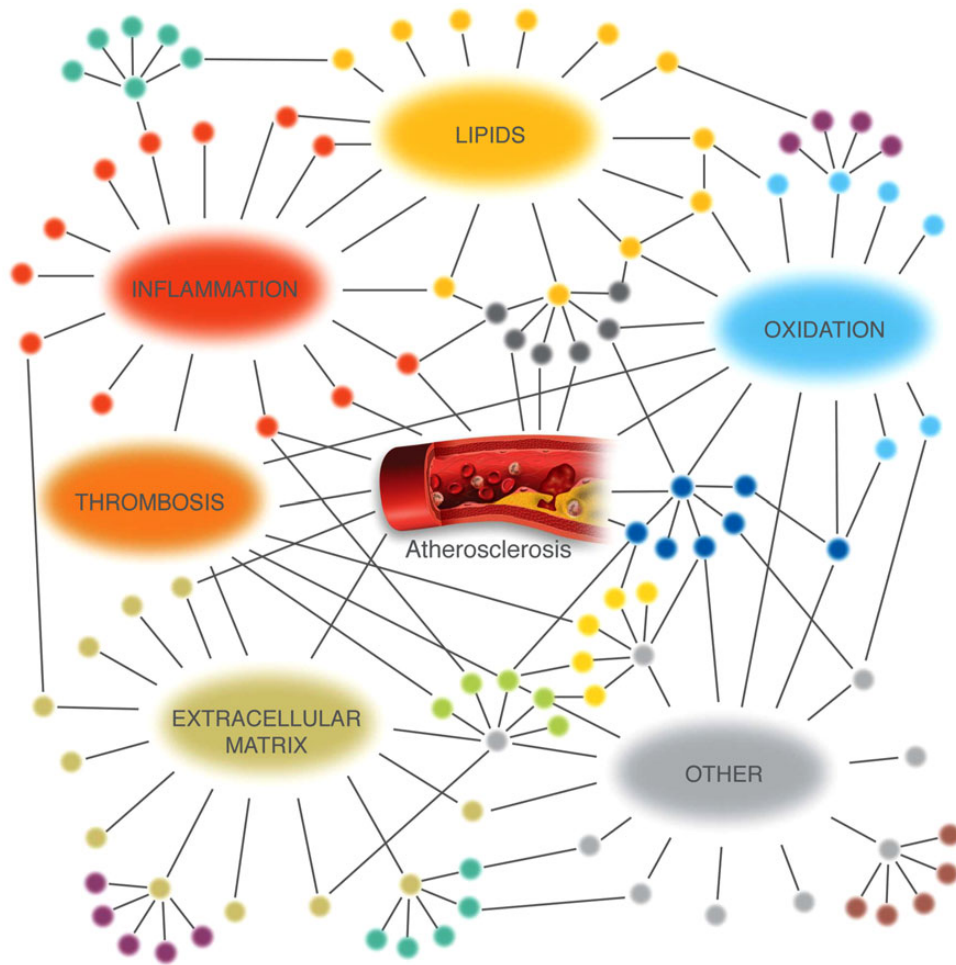


Figure 2 Interacting nodes and networks, not linear pathways, may operate during the pathogenesis of atherosclerosis. An alternative concept suggested by the systems biology approach postulates nodes or clusters of pathogenesis that interact in complex and multidimensional ways not taken into account by our traditional linear constructs. The nodes may constantly vary, adjusting for changing conditions and trying to maintain homeostasis and respond to various endogenous or environmental stimuli.

Ultimately, translating the results of mouse studies to treatments of the human disease will require large-scale clinical trials, showing that the novel therapy can actually improve outcomes in a net beneficial manner over unwanted effects. Well-designed experimental studies such as the one presented by Petri inform the quest for targets that may ultimately help confront the unmet need of addressing the residual burden of cardiovascular disease that persists despite the excellent current treatments in our armamentarium.

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Reference

1. Petri MH, Laguna-Fernandez A, Gonzalez-Diez M, Paulsson-Berne G, Hansson GK, Back M. The role of the FPR2/ALX receptor in atherosclerosis development and plaque stability. *Cardiovasc Res* 2015;**105**:65–74.