Title

Aneurysms: leukotrienes weaken aorta from the outside

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induces thyroiditis but does not lead to hypothyroidism.

Quaratino *et al.*[10] generated a transgenic animal that overcomes many of these limitations. The mice are transgenic for the T-cell receptor (TCR) genes of a TPO-specific T-cell clone isolated from the thyroid gland of an individual with thyroiditis. The clone recognizes a TPO peptide generated by processing in thyroid cells or TPO-expressing B cells. Because T cells recognize peptides presented by histocompatibility leukocyte antigen (HLA)/major histocompatibility class (MHC) molecules and the clone is of human origin, splenocytes from mice with different MHCs were tested to find a strain able to present this peptide effectively to transgenic T cells.

The peptide recognized by the TPO-specific T-cell clone interacted similarly with the mouse and human HLA. Moreover, the human peptide and its mouse homolog have high sequence similarity, explaining how T cells from the mice interact with mouse TPO and cause disease. The mice express the human TCR on $CD4^+$ and $CD8^+$ T cells; T-cell activation was spontaneous and $CD8^+$ T cells killed target cells loaded with the TPO peptide. To prevent interference from endogenous TCRα chains, the transgenic line was backcrossed to recombinase-activating gene–deficient ($RAG^{-/-}$) mice. Virtually all TAZ10 mice (TCR-transgenic $RAG^{-/-}$ animals) spontaneously developed thyroiditis and hypothyroidism.

This elegant model addresses several major questions. The study clearly shifts the emphasis away from a role for autoantibodies in thyroid damage, and shows that TPO-specific T cells alone can produce thyroid destruction leading to hypothyroidism. Whether $CD8^+$ T cells or cytokines released by $CD4^+$ T cells mediate thyrocyte damage remains to be determined. Also notable is the finding that thyroiditis developed spontaneously in these mice without the need for immunization with the TPO peptide. This study supports one intriguing hypothesis[11]—that T-cell epitopes that are hidden ('cryptic') during thymic education and later expressed in the periphery (thyroid gland, in this model) are involved in autoimmunity. Although in this animal model TPO antibodies clearly do not damage thyrocytes, in human disease TPO-specific B cells or autoantibodies, or both, may be pivotal in presenting TPO to T cells, thereby playing an indirect role in thyroid damage.

Thyroiditis and hypothyroidism developed in almost all of the TCR transgenics on the $RAG^{-/-}$ background but in only half of those on the $RAG^{+/+}$ background. This observation suggests that the T-cell clone used to generate the transgenic mice may only have weak cytotoxic activity that was 'diluted' by non-TCR-transgenic T cells in the $RAG^{+/+}$ mice. On the other hand, the authors mention that they have preliminary evidence for regulatory T cells in $RAG^{+/+}$ TCR transgenics that could protect against thyroid destruction. Expanding regulatory T cells in these animals could provide an immunological approach to preventing thyroid damage with implications for other organ-specific autoimmune diseases such as type 1 diabetes.

The model also raises questions. Which cells present TPO to the transgenic T cells? Are thyroid cells involved in antigen presentation and do they express MHC class II as in human thyroid autoimmunity? Does the autoimmune response spread from TPO to thyroglobulin after thyroid damage and release of tissue-specific proteins? Finally, can the model provide insight into why some patients with autoimmune thyroiditis 'compensate' and remain euthyroid whereas others develop hypothyroidism? In this regard, backcrossing TAZ10 mice to mouse strains with the same MHC but with different background genes would make it possible to analyze the contribution of non-MHC genes to thyroid damage. Such novel genes would provide invaluable candidates for investigation in the multigenic setting of human thyroiditis.

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Aneurysms: leukotrienes weaken aorta from the outside

Wulf Palinski

5-lipoxygenase, a key enzyme in the formation of proinflammatory leukotrienes, is upregulated in the macrophages of the outer layer of the aorta and promotes aneurysm formation in mice fed a high cholesterol diet. Could this point to nonsurgical prevention of aortic aneurysms (pages 966–973)?

The mechanisms responsible for the formation of arterial aneurysms remain mysterious. Key features include extensive inflammation of the adventitia, the outermost layer of arteries, and dilation of the media, the smooth muscle cell–rich middle layer that conveys mechanical stability and elasticity. Progressive weakening of the media may ultimately result in arterial rupture, often giving rise to sudden death. Aneurysms are also frequently, but not always, associated with atherosclerosis, a chronic inflammatory condition causing thickening of the intima and compensatory dilation of the media. We do not know whether this association is merely coincidental or whether the two processes are pathogenically linked.

Prevention of life-threatening acute ruptures of the abdominal aorta, the most frequent site of aneurysms (**Fig. 1**), is essentially limited to prophylactic surgery. This is not without risk and is therefore mainly performed when the abdominal aorta exceeds 5.5 cm in diameter or when accelerated dilation occurs. Disappointingly, elective surgery of 'early' aneurysms (greater than twofold dilation, but smaller than 5.5 cm) does not improve survival[1]. Interventions with drugs that target key pathogenic factors of aneurysm formation would therefore be highly desirable. In this issue, Zhao *et al.* identify one such

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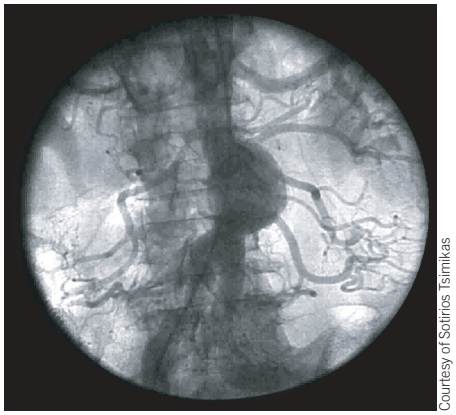

Courtesy of Sotirios Tsimikas

Figure 1. Angiogram of an abdominal aortic aneurysm from an 80 year old, currently asymptomatic male with a history of hypertension, diabetes mellitus, hypercholesterolemia, smoking, and myocardial infarction. The movie (**Supplemental Movie 1** online) shows a heavily calcified, atheromatous aneurysm that fills with swirling contrast media.

factor, 5-lipoxygenase (5-LO) expressed by adventitial macrophages, and investigate how it may promote inflammation and aneurysm formation[2].

5-LO, the key enzyme in leukotriene production[3], is expressed in polymorphonuclear leukocytes, macrophages and mast cells. Upon activation of these cells, 5-LO translocates from the cytoplasm to the nuclear envelope and, together with the cofactor 5-lipoxygenase-activating protein (FLAP), initiates production of leukotrienes from arachidonic acid freed from cell membranes. The initial product, LTA_2, is then converted to either LTB_4 or LTC_4, which may undergo further extracellular metabolism to LTD_4 and LTE_4. The three cysteinyl leukotrienes LTC_4, LTD_4, and LTE_4 are potent mediators of inflammation and together constitute what is known as the slow-reacting substance of anaphylaxis.

Since the biochemistry of eicosanoids—arachidonic acid derivatives including prostaglandins and leukotrienes—was first elucidated, the field has gone through repeated cycles of euphoria and disappointment[3]. Lately, renewed interest in leukotrienes has been triggered by the observations that 5-LO, LTD_4 and LTE_4 are expressed in arteries[4] and that polymorphisms of 5-LO promoters and FLAP haplotypes correlate with risk of coronary heart disease[5]. A small study even suggested that 5-LO promotes atherogenesis in mice[6].

Zhao *et al.*[2] performed a comprehensive investigation of the role of 5-LO in atherogenesis and aneurysm formation in several mouse models and human tissues. Contrary to immunohistochemical analysis of human arteries[4], 5-LO-expressing macrophages were virtually absent from the intima of apoE knockout mice (one of the most widely used models of atherosclerosis), irrespective of the stage of atherosclerosis. In contrast, 5-LO–expressing macrophages abounded in the adventitia. Moreover, expression of 5-LO in macrophages increased with atherosclerosis, as did expression of its cofactor, FLAP, and two G-protein-coupled receptors for LTD_4 ($CysLT_1R$ and $CysLT_2R$). Administration of a hypercholesterolemic diet supplemented with the proinflammatory agent cholate to apoE knockout mice induced extensive adventitial granulomas and frequent aortic aneurysms. In mice null for the gene encoding 5-LO as well as apoE, the authors found a greatly reduced incidence and severity of aneurysms compared to the apoE knockout mice, but no significant changes in the extent of atherosclerosis. This is consistent with the observation that 5-LO was not expressed in atherosclerotic lesions of mice. These observations indicate that the 5-LO–leukotriene pathway is selectively activated in the adventitia and that adventitial inflammation is a major cause of aneurysm formation.

The authors also began to dissect how 5-LO may promote inflammation and aneurysm formation. Disruption of 5-LO reduced the circulating level of macrophage inflammatory protein 1α (MIP-1α or CCL3) and microarray analysis of human cells confirmed that the 5-LO–LTD_4 pathway upregulates MIP-1α in macrophages and MIP-2/CXCL2 in endothelial cells.

These results support the notion that activation of macrophages in the adventitia triggers 5-LO expression and that this greatly enhances the recruitment and activation of macrophages and T cells by paracrine and endocrine mechanisms (**Fig. 2**). Secretory products of activated macrophages, such as metalloproteinases, would then weaken the media[7]. The arterial activity of one metalloproteinase, MMP-2, was indeed increased in the present study, albeit not as a direct effect of 5-LO.

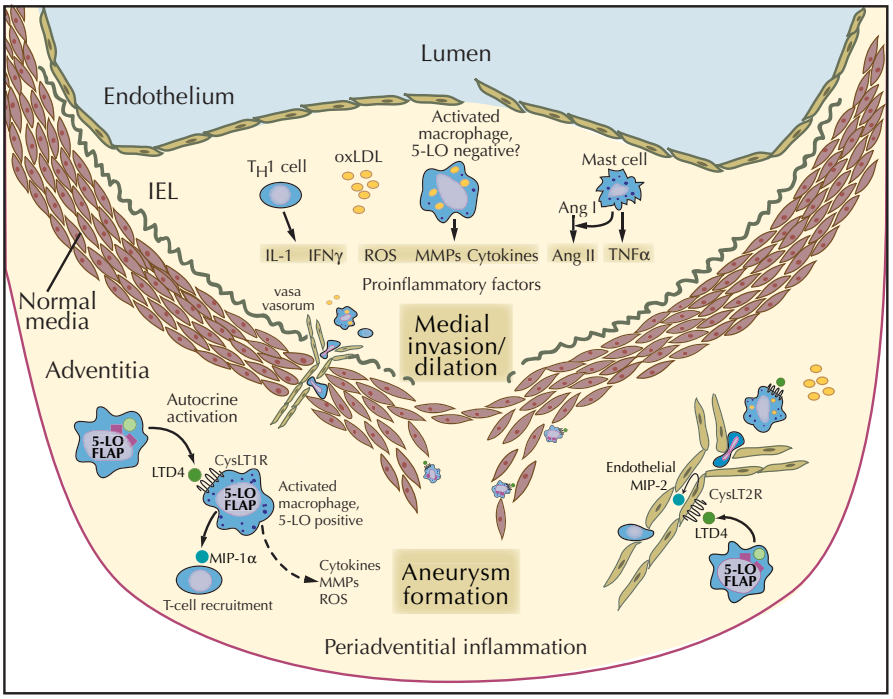

Figure 2 Aortic remodeling and aneurysm formation. Zhao *et al.* provide evidence that adventitial macrophages express 5-LO and its cofactor, FLAP, and generate leukotrienes, which set in motion a number of proinflammatory events. One of the leukotrienes, LTD_4, causes autocrine activation of macrophages through binding to $CysLT_1$ receptors. Increased leukotriene formation also promotes the recruitment of monocytes—the precursors of tissue macrophages—and T cells. Specifically, LTD_4 binds to $CysLT_2$ receptors on endothelial cells of the many microvessels present in the adventitia and media (vasa vasorum), resulting in increased endothelial release of MIP-2 and leukocyte extravasation. Activated macrophages also release MIP-1α, which may further promote T cell recruitment. Independent of the 5-LO pathway, activated macrophages generate other proinflammatory factors, including metalloproteinases (MMPs), that weaken the media. Atherosclerosis in the intima may act synergistically with adventitial inflammation. Intimal macrophages, T_H1 cells and mast cells secrete many proinflammatory factors, including IFNγ, IL-1, MMPs and TNFα. Mast cells may also contribute to the conversion of angiotensin I to angiotensin II, a powerful promoter of aneurysms in mice[9]. Hypercholesterolemia is an essential cofactor of both adventitial and intimal inflammation.

How relevant are these findings for human aneurysm formation? The most obvious caveats concern mouse models of cardiovascular diseases in general[8]. These include differences in lipoprotein metabolism, confounding influences of the underlying genetic defects, the thin media consisting of just a few smooth muscle cell layers, the morphology of fibrous caps, the rarity of spontaneous plaque rupture and subsequent thrombus formation, immunological differences (e.g., the T_H1 bias of C57BL/6 mice), and the frequent and early invasion of mouse lesions into the media. Nevertheless, these models reflect many characteristics of human atherosclerosis and are invaluable for the investigation of pathogenic mechanisms, including those leading to medial remodeling and plaque vulnerability. Needless to say, results obtained in any animal model should never be extrapolated to humans without confirmation by histopathology or clinical trials.

There are also some unresolved issues, such as why only adventitial macrophages express 5-LO. Clearly, this cannot be due to systemic factors, such as hypercholesterolemia or circulating MIP-1α. The absence of 5-LO expression in the intima also conflicts with observations in human arteries[5], and the lack of an effect of 5-LO deficiency on atherogenesis is counterintuitive, given the powerful proinflammatory effects of 5-LO. These caveats do not, however, reduce the importance of the results reported here.

Elucidation of the mechanisms of aneurysm formation is greatly hindered by the lack of a universally accepted animal model. Many interventions cause medial dilation, including elastase perfusion, adventitial calcium chloride and mechanical injury[9]. More physiologically, angiotensin II overexpression results in dramatic mouse aneurysms independent of vasopressory effects[9]. The common denominator of these interventions, and a prominent element in human aneurysms, is adventitial inflammation. The present study mimics the adventitial events in human aneurysms, but also highlights another essential point: the need for proinflammatory cofactors. In models without cofactors, adventitial inflammation and resulting medial and intimal lesions are often transitory. A necessary cofactor in this study, and probably the most important one in humans, is hypercholesterolemia, which promotes lipid peroxidation, affects oxidation-sensitive regulation of gene expression, triggers immune responses to antigens (e.g. oxidized LDL) and increases leukocyte recruitment[10]. The necessity in the present study to further enhance inflammatory conditions with cholate reinforces this concept.

It should not be forgotten that hypercholesterolemia is also a major cause of atherosclerosis, that similar proinflammatory events occur in the intima, and that atherosclerosis is inherently associated with compensatory medial enlargement. Furthermore, vasa vasorum—microvessels originating from the adventitia that cross the media and reach deeper layers of atherosclerotic lesions—contribute to the progression of atherosclerosis[11], but also constitute a prime avenue for intimal-adventitial interactions. It is therefore likely that atherosclerosis provides an important inflammatory boost and that aneurysms, at least in the human abdominal aorta, result from synergistic attacks from both the adventitia and intima (**Fig. 2**).

The present results suggest that 5-LO inhibitors or $CysLT_1$ receptor antagonists similar to those used to treat asthma may inhibit aneurysms. But in view of the likely contribution of intimal proinflammatory factors, combined COX and leukotriene inhibition may be more effective. The role of hypercholesterolemia in aneurysms also bolsters the case for statins, which may have the added benefit of inhibiting chronic inflammation[12]. In light of the recent insights, the concept that surgery is the only therapy for patients with small aneurysms should be revisited and clinical trials investigating the effectiveness of new anti-inflammatory treatment may be indicated.

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Epithelial cells pay a Toll for protection

Warren Strober

Ligands from commensal microflora can stimulate Toll-like receptors on the surface of intestinal epithelial cells. Work in mice now assigns a surprising role to such signals: cell survival and repair during inflammation.

Toll-like receptors (TLRs) recognize general features of microorganisms, such as cell-wall lipids, peptidoglycans and stretches of guanine oligonucleotides[1]. This feature enables a swift immune response to invaders but creates the danger of over-reaction to commensal microorganisms, particularly in cells that are constantly in proximity to them, such as the epithelial cells lining the lower intestinal tract. One mechanism for dealing with this problem is reduced responsiveness to repeated TLR signaling in epithelial cells[2,3]. In a recent issue of *Cell*, Rakoff-Nahoum *et al.* qualify this mechanism, finding that epithelial responsiveness to TLR is probably not irreversibly throttled down[4]. They report that robust TLR signaling via commensal organisms occurs during inflammation and other insults and protects epithelial cells in mice from injury.

That commensals modulate TLR signaling is best shown by studies indicating that signaling through TLR4 or TLR2 in epithelial cells *in vitro* readily occurs after initial exposure to ligand[3]—but not after second exposure or prolonged incubation with ligand. This downregulation of signaling occurs along with decreased receptor expression on the cell surface and increased

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