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Folate Receptor: A Macrophage “Achilles’ Heel”?

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Cardiovascular diseases are the most common cause of death worldwide.¹ Atherosclerosis, the underlying inflammatory disease, develops through the progressive accumulation of lipids and leukocytes in the vessel wall. Over the past 30 years, lesional macrophages have emerged as central inflammatory orchestrators of disease progression and its complications. Macrophage precursors, the circulating monocytes, invade predilection sites of dysfunctional endothelium, differentiate, and engulf lipids. Lesional macrophages secrete cytokines, chemokines, growth factors, and lytic enzymes that promote plaque destabilization and rupture, which lead to myocardial infarction and stroke. Interference with monocyte recruitment and macrophage accumulation reduces lesion burden in experimental models, which suggests that such approaches could be beneficial in human disease.^{2–4}

Much of current cardiovascular disease management focuses on treatment of known risk factors such as high cholesterol. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, the class of drugs known as statins, lower cholesterol but also can exert atheroprotection by targeting inflammation.^{5,6} Treatment of atherothrombotic disease by direct and specific targeting of inflammation is being investigated,⁷ and clinical trials with low-dose methotrexate⁸ and anti-interleukin-1 β ⁹ are under way. Although these studies are exciting, previous work reminds us that antiinflammatory approaches require caution. Indiscriminate blockade of CD40L showed promise in several experimental studies, but clinical trials had to be aborted because of thromboembolic complications. More selective inhibition of the interaction of CD40L with integrin

Mac-1, however, impaired atherogenesis without affecting thrombus formation.¹⁰ The example illustrates that strategies aimed at targeting inflammation should be considered in their immunophysiological and molecular contexts.

In this issue of the *Journal of the American Heart Association*, Furusho et al¹¹ endeavor to treat atherosclerosis by targeting and killing lesional macrophages with an anti-folate receptor β (FR β) immunotoxin. Macrophages upregulate FR β during inflammation.¹² Peritoneal murine macrophages, for example, upregulate FR β in response to challenge with thioglycollate, zymosan, or bacteria,¹³ and macrophages in rheumatoid arthritis and pulmonary fibrosis express functionally active FR β that can be targeted therapeutically in an experimental setting.^{14–17} The idea for the study by Furusho et al was therefore simple. If lesional FR β -expressing macrophages are atherogenic, then delivery of a recombinant immunotoxin (a truncated *Pseudomonas* exotoxin A [PE38] conjugate that kills the infected cell) to those macrophages should be atheroprotective. The anti-FR β immunotoxin was meant to achieve 3 aims: to target FR β -expressing cells, to facilitate internalization of the toxin in those cells, and, by implication, to minimize adverse effects of the toxin on bystander, non-FR β -expressing cells.

Although others have shown in animal models that lesional macrophages upregulate the folate receptor,^{18,19} Furusho et al are the first to target increased FR β expression on lesional macrophages therapeutically. The authors provide evidence by immunohistochemistry and immunofluorescence that FR β is expressed by 60% to 70% of CD68-positive lesional macrophages but not by T cells, endothelial cells, or smooth muscle cells. The authors report on FR β expression in human carotid artery plaques and show that treatment of mice with anti-FR β immunotoxin decreased lesional macrophage content compared to controls. This reduction in cell number was associated with a reduction in percent lesion area. Importantly, differential blood cell counts did not change in the anti-FR β immunotoxin group, which suggests a mechanism that is localized and presumably limited to the plaque. Indeed, the authors reported increased cell death in lesions, lending further support that the anti-FR β immunotoxin acted locally.

Is the study convincing? The effects of the immunotoxin on CD68-positive macrophages are striking, but it is not yet

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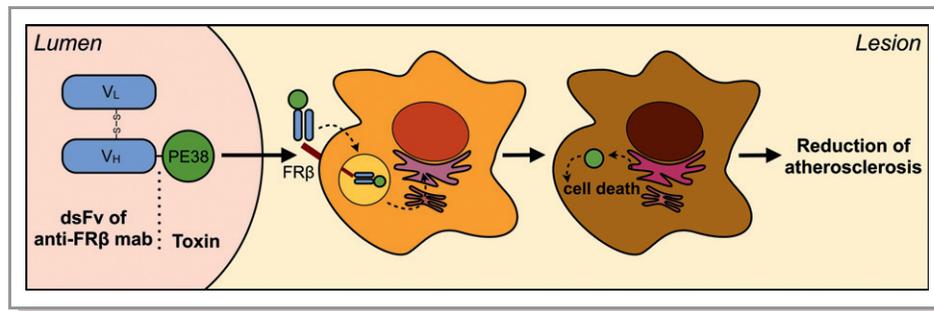


Figure 1. Proposed model of immunotoxin-mediated killing of $FR\beta$ -expressing macrophages in atherosclerotic lesions. The immunotoxin consists of a recombinant, biologically active fragment of *Pseudomonas* exotoxin A (PE38) that is linked to the disulfide-stabilized variable region fragment (dsFv) of a monoclonal antibody (mab) against $FR\beta$. Upon binding to $FR\beta$ on lesional macrophages, the immunotoxin is internalized and intracellularly processed, leading to toxin-mediated adenosine triphosphate-ribosylation and inactivation of elongation factor 2, inhibition of protein synthesis, and cell death. Elimination of $FR\beta$ -positive macrophages reduces atherosclerosis. V_L indicates variable region of the light chain; V_H , variable region of the heavy chain; and $FR\beta$, anti-folate receptor β .

entirely clear how the immunotoxin achieves this. The TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) staining is a clue that the immunotoxin specifically triggers cell death, but no colocalization of TUNEL-positive and $FR\beta$ -expressing cells is shown. Moreover, a precise phenotypic analysis of $FR\beta$ -expressing cells in atherosclerotic lesions is lacking, and the observation that CD68-positive cells colocalize with $FR\beta$ does not preclude the possibility that other important cells are affected.

Despite some uncertainties, the phenotype described in this work is compelling and warrants further work. It remains to be determined whether $FR\beta$ -expressing macrophages represent a distinct subset of lesional macrophages or an activation state, as well as whether these macrophages are functionally distinct from their $FR\beta$ -negative counterparts. As the authors mention, induction of macrophage apoptosis in atherosclerotic lesions could be a double-edged sword. Although beneficial in early stages of lesion formation, the increased cell death at more advanced stages might be detrimental.²⁰ It is also worth considering that folic acid and folate, also known as vitamin B9, are important to many biological functions, such as DNA synthesis and repair. Elimination of cells expressing a particular receptor could have other unforeseen effects that counterbalance the potential benefits observed in this study. Nevertheless, the authors have engineered a clever method to target a formidable cell (Figure 1). The Greek metaphor, though imperfect, can be illustrative: If the macrophage is Achilles and the folate receptor is his Heel, then the antibody is Paris' arrow and the exotoxin the poison that ultimately kills the greatest warrior of the Trojan War.

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Disclosures

None.

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