

Treatment of Inflammation and Atherogenesis

Therapeutic Targeting of CD40L/Mac-1 interaction

Technology

CD40L participates in chronic inflammatory diseases such as atherosclerosis. Through interaction with its classic receptor CD40, CD40L regulates B-cell and T-cell function. CD40L also stabilizes thrombi through interaction with the platelet integrin $\alpha_{IIb}\beta_3$. While anti-CD40L antibody treatment generated promising results in early clinical trials, elevated thrombembolic complications prohibited the pursuit of this strategy. In addition, long-term inhibition of CD40L - as is most likely required for treatment of chronic inflammatory diseases - severely compromises host defenses, rendering generalized inhibition of CD40L an unappealing treatment strategy.

We previously reported that CD40L mediates atherogenesis independently of CD40 in mice, and proposed a novel interaction with the leukocyte integrin Mac-1. We have characterized this interaction on a molecular level, identifying the amino acids E¹⁶²-L¹⁷⁰, located on an exposed loop between the $\alpha 1$ helix and β -sheet B of the Mac-1 I-domain, as a distinct binding site for CD40L. Targeting of CD40L/Mac-1 binding with a stable inhibitory peptide, cM7, proved specific and ultimately effective in attenuating inflammation and atherosclerotic lesion formation in mice. Specific inhibition of the CD40L/Mac-1 interaction might therefore represent an attractive novel anti-inflammatory treatment strategy for atherosclerosis and other chronic inflammatory diseases, avoiding the unwanted effects of global inhibition of CD40 ligand action.

Innovation

- Our peptide cM7 is efficacious and specific in the inhibition of CD40L/Mac-1 binding and its downstream effects, such as inflammatory gene expression, inflammatory cell recruitment and atherogenesis.
- Previous concepts aimed at global inhibition of cytokines such as CD40L largely failed due to acute or long-term side effects.
Our peptide-based strategy might overcome some of these limitations.

Application

- Anti-inflammatory treatment for atherosclerosis and other chronic inflammatory diseases

Market Potential

- Atherosclerosis, as a prevalent disease among people in the modern world, causes disabilities and a high mortality rate.

Responsible Scientist

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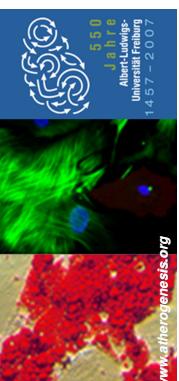
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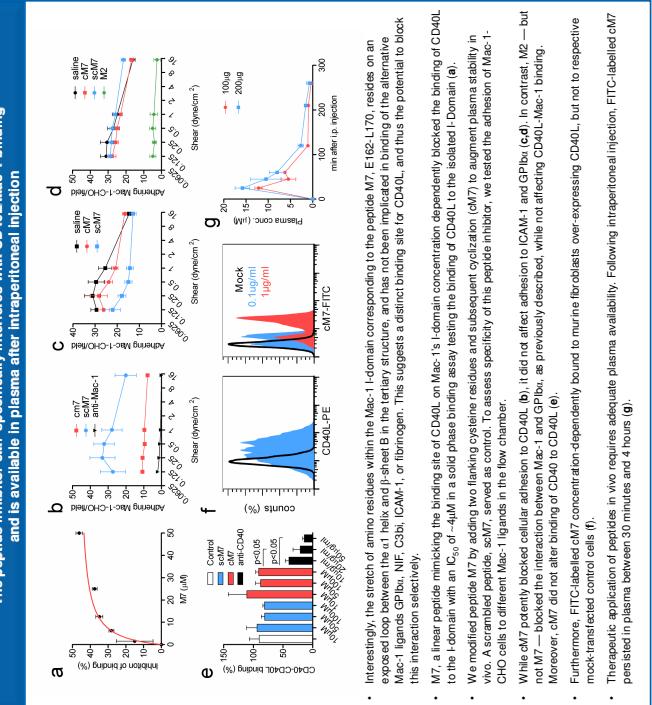
Selective Targeting of the CD40L/Mac-1 Interaction by a Small Peptide Inhibitor Limits Inflammation and Atherogenesis in Mice

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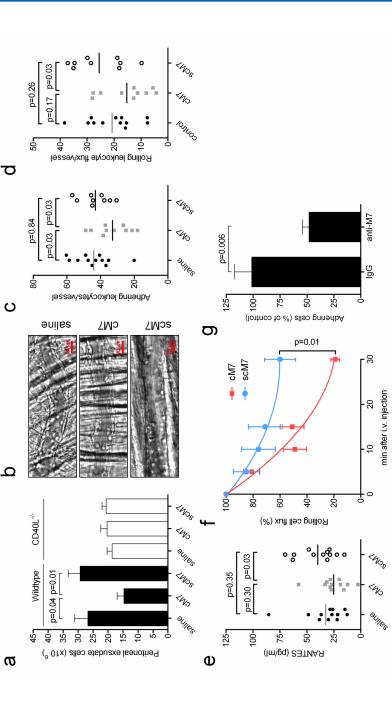
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The peptide inhibitor cm7 specifically interferes with CD40L/Mac-1 binding and is available in plasma after intraperitoneal injection

Specific blockade of the CD40L/Mac-1 interaction attenuates atherosclerosis in mice



cm7 attenuates inflammatory leukocyte recruitment in vitro and in vivo



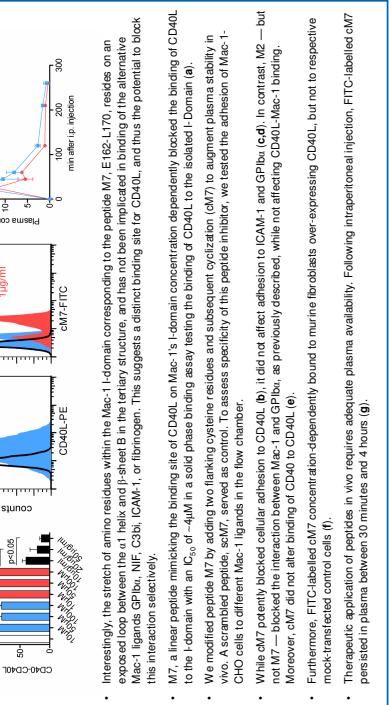
Conclusion

- While inflammation drives many chronic diseases, including atherosclerosis, few selective anti-inflammatory treatment options currently exist.
- In the context of atherosclerosis, statins allow a glimpse at the therapeutic potential of such strategies. Another class of drugs, the Cox-2 inhibitors, exemplifies the impressive extend of therapeutic benefits but they also demonstrate the difficulty in developing anti-inflammatory drugs without side effects.
- Beyond a mere reduction in size, atherosclerotic plaques from cm7-treated animals contained significantly fewer macrophages and lower lipid accumulation, while smooth muscle cells increased compared with the appropriate peptide control group, as assessed by immunohistochemistry in sections of the aortic root (see).
- We therefore tested whether our peptide inhibitor could mitigate atherosclerosis *in vivo* in mice.
- LDLR^{-/-} mice consuming a high-cholesterol diet for 20 weeks developed significantly smaller lesions both in the aortic sinus and abdominal aorta when treated with cm7 (ab).
- Previous concepts aimed at the global inhibition of cytokines such as IL-10, IL-12, or TNF-α — upon long term treatment with cm7 — indicated a Th1-Th2 phenotype — such as IL-10, IL-12, or TNF-α — upon long term treatment with cm7 did not show, nor in T-cell content or proliferation in the plaque (h, i). Lipid levels, weight, leukocyte subsets, blood pressure, cytokine levels, and chemokine levels remained unchanged (data not shown).
- Therefore, cm7 may represent a fruitful novel strategy to combat chronic inflammatory diseases such as atherosclerosis.

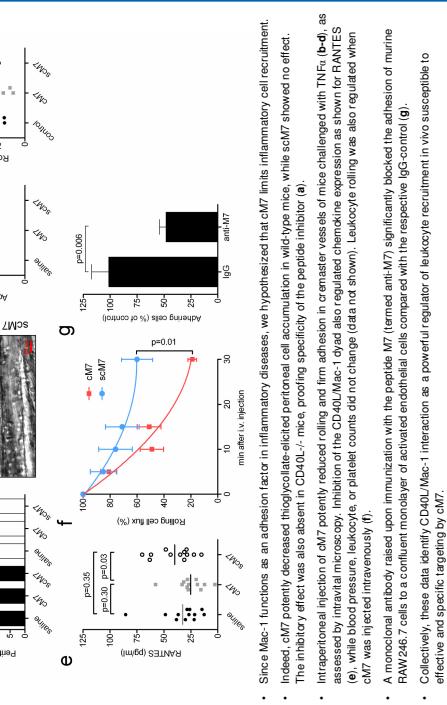
Background

- CD40L participates in chronic inflammatory diseases such as atherosclerosis. Through interaction with its classic receptor Integrin $\alpha_4\beta_1$.
- While anti-CD40 antibody treatment generated promising results in early clinical trials, elevated thrombotic events — as most likely required for treatment of chronic inflammatory diseases — severely compromised host defenses, rendering generalized inhibition of CD40L an unsatisfactory treatment strategy.
- We previously reported the CD40L mediates atherosclerosis independently of CD40 in mice, and proposed a novel interaction with the leukocyte integrin Mac-1. Here we characterized this interaction, designed an inhibitory peptide, and tested whether this peptide limits inflammation and atherosclerosis in mice.

Endothelial CD40L recruits inflammatory leukocytes in interaction with Mac-1



A peptide mapping strategy identifies a distinct binding site for CD40L on Mac-1



- Since Mac-1 functions as an adhesion factor in inflammatory diseases, we hypothesized that Mac-1 is a binding partner for CD40L.
- Recombinant variants of the I-domain and CD40L were produced in a bacterial expression system.
- In a solid phase binding assay, CD40L, either soluble immobilized, specifically bound to the isolated I-domain (b), A KD of 66nM revealed a high-affinity interaction.
- To identify the binding site used by CD40L, we employed a peptide mapping strategy using linear peptides M1-M8, originating from the hydroxyl surface of the Mac-1 domain.
- In an initial solid phase binding assay, evaluating the binding of the isolated Mac-1 I-domain to immobilized CD40L, the Mac-1 fragments M3, M4, M5, and M6 emerged as potential binders (e).
- In the more physiologically setting with the entire Mac-1 protein in a cell membrane environment, M7 most efficiently blocked adhesion of THP-1 cells to CD40L.
- Moreover, M7 was the only peptide blocking binding of CD40L to human granulocytes and monocytes in flow cytometry (e).
- Most of Mac-1's ligands — such as fibrinogen, ICAM-1, CIPB, RAGE, C3b, or heparin — bind to the Mac-1 I-domain, a stretch of ~200 amino acids within the I-domain of the integrin (a), we hypothesized that the I-domain also serves as binding partner for CD40L.
- Recombinant variants of the I-domain and CD40L were produced in a bacterial expression system.
- In a solid phase binding assay, CD40L, either soluble immobilized, specifically bound to the isolated I-domain (b), A KD of 66nM revealed a high-affinity interaction.
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- Moreover, M7 was the only peptide blocking binding of CD40L to human granulocytes and monocytes in flow cytometry (e).
- A monoclonal antibody raised upon immunization with the peptide M7 (termed anti-M7) significantly blocked the adhesion of murine RAW264.7 cells to a confluent monolayer of activated endothelial cells compared with the respective IgG control (g).
- Collectively, these data demonstrate that a pan-I-domain blocking antibody (g).