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Complement regulator CD59 protects against angiotensin II-induced abdominal aortic aneurysms in mice

Gongxiong Wu (MD/PhD)[a,b], Ting Chen (PhD)[a,b], Weiguo Hu (MD/PhD)[a,b], Guo-Ping Shi (PhD)[a], Huseyin Aktas (PhD)[a,b], Jose A. Halperin (MD)[a,b], Xuebin Qin (MD/PhD)[a,b]

[a] *Department of Medicine, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA*
[b] *Harvard Medical School, Laboratory for Translational Research, One Kendall Square, Building 600, 3rd Floor, Cambridge, MA 02139, USA*

Mediators of immunity and inflammation play a critical role in the pathogenesis of aneurysm including abdominal aortic aneurysm (AAA). The complement is a main effector of the immune system and mediators of inflammation. The complement system is activated by three activation cascades, which lead to formation of the membrane attack complex (MAC). MAC is a macromolecular pore capable of inserting itself into cell membranes and lysing heterologous cells and bacteria, and an important mediator of cellular signals including the nuclear factor NF-κB, and the activator protein-1 (AP-1) that trigger mitogenic effects. The immune histological analysis of human aneurysm indicates that complement and MAC may play a role in the pathogenesis of aneurysm. However, the role of MAC in the aneurysm pathogenesis has not been extensively investigated in animal model. Here, we evaluated the pathogenic roles of MAC and CD59, a key regulator that inhibits MAC, in the development of AAA. We demonstrated that in the angiotensin II-induced AAA model, deficiency of MAC regulator CD59 in ApoE-null mice (mCd59ab−/−/ApoE−/−) accelerated the disease development, while transgenic over-expression of human CD59 (hCD59ICAM-2+/−/ApoE−/−) in this model attenuated progression of AAA. The severity of aneurysm among these three groups positively correlates with C9 deposition, and/or the activities of matrix metalloproteinase 2 (MMP2) and MMP9, and/or the levels of phosphor (p)-c-Jun and p-c-Fos, indicative of AP-1 signaling pathway activation; and p-IKK-α/β and p-65, indicative of NF-κB signaling pathway activation. Furthermore, we demonstrated that MAC directly induced gene expression of MMP2 and MMP9 in vitro, which required activation of AP-1 and NF-κB signaling pathways. Together, these results defined the protective role of CD59 and shed light on the important pathogenic role of MAC in AAA.

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High throughput screening of small molecule inhibitors of human alternative pathway complement

Yuko Kimura[a], Chun-Hao Chiu[b], Andrew D. Napper[b], Scott L. Diamond[b], Wen-Chao Song[a]

[a] *Department of Pharmacology and Institute for Translational Medicine and Therapeutics, University of Pennsylvania, Philadelphia, PA 19104, United States*
[b] *Institute for Medicine and Engineering and Department of Chemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia, PA 19104, United States*

The alternative pathway (AP) plays a critical role in complement-mediated human diseases. Much effort has been made to develop specific inhibitors for human complement and recent works with several biological reagents (mAbs and recombinant proteins) have provided evidence that it is feasible to selectively inhibit the AP without compromising other complement pathways. We explored the use of high throughput screening (HTS) to identify potential small molecule inhibitors of AP complement. We adapted and optimized the LPS-based Wielisa test of AP complement activation into a 384-well ELISA plate format compatible with automated robot-assisted HTS. Using 10% normal human serum (NHS) and an orthogonal mixture strategy, we identified 52 "hits" in a first-round screening of 62,139 compounds from the National Institutes of Health Molecular Libraries Small Molecule Repository. These compounds were subjected to a second-round screening and one lead compound was confirmed to inhibit human AP complement dose-dependently with an IC50 of $0.40 \pm 0.07\,\mu M$. Further testing with 10% NHS and LPS-coated ELISA, zymosan C3 deposition and rabbit erythrocyte lysis assays validated the lead compound as an AP complement inhibitor. Preliminary biochemical studies using purified complement components showed that the lead compound inhibited the activity but not the formation nor promoted the decay of the AP C3 convertase C3bBb, with Bb as the most likely target. AP complement-inhibiting activity by the lead compound was also demonstrated in 25% NHS but not at 50% or higher NHS. Our experiments demonstrate the feasibility of HTS in identifying chemical inhibitors of complement in a single assay with multiple AP components as potential targets. With further optimization of the HTS protocol (e.g. using more concentrated NHS) and computer-aided modeling of candidate compounds, it may be possible to discover more potent and selective small molecule inhibitors of human AP complement.

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C4B*Q synergizes with smoking to precipitate chronic obstructive pulmonary disease

E.T. Bjornsson[a,b,c], K. Einarsdottir[a,b,c], B. Benediktsdottir[a,b,c], G. Gudmundsson[a,b,c], Th. Gislason[a,b,c], G.J. Arason[a,b,c]

[a] *Department of Immunology, Landspitali University Hospital, Hringbraut, Reykjavik, Iceland*
[b] *Department of Medicine, University of Iceland, Reykjavik, Iceland*
[c] *Department of Medicine, Landspitali University Hospital, Hringbraut, Reykjavik, Iceland*

Background: We previously demonstrated that C4B*Q0 is inconsistent with health for smokers (Arason et al., 2007). The carrier frequency of C4B*Q0 drops from 19.2% through 4% to 0% in healthy Icelandic smokers at ages 50 and 60, respectively. In search for a reason, we analyzed individuals with suspected cardiovascular disease (CVD), and found that C4B*Q0 was markedly raised in smokers with their first lifetime myocardial infarction (MI) ($p = 0.0003$, odds ratio (OR) 22.66) and also in smokers with angina pectoris (AP) ($p = 0.005$, OR 30.07). Moreover, smokers with C4B*Q0 had a markedly decreased chance for survival after MI (hazard ratio 3.50, $p = 0.008$) (Blaskó et al., 2008).

Rationale: C4B*Q0 is inconsistent with health for smokers and this may be due to their high risk of developing CVD. However, other diseases affecting the middle aged should also be studied.

Methods: We analyzed C4B*Q0 carrier frequencies in a population sample of 412 Icelanders, using high-voltage agarose electrophoresis (HVAGE). Patients with CVD were excluded from the study. The results were correlated with smoking status and with diagnosis of chronic obstructive pulmonary disease (COPD) stage II.

Results: Carrier frequencies of C4B*Q0 dropped from 20.7% to 14.42% after age 50 ($p = 0.067$) and when the analysis was confined to smokers, the frequencies dropped from 17.2% to 9.6% ($p = 0.109$).