Low mannose-binding lectin levels predict cardiovascular disease in hemodialysis patients

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Published in:
Immunobiology

DOI:
10.1016/j.imbio.2016.06.132

Publication date:
2016

membranes by forming dimers, thus enhancing their function as competitors of FH. Plasma concentrations of FHR-1, FHR-2 and FHR-5 have not yet been established, as the dimer composition has prevented their detailed characterization to date.

We immunized mice with recombinant (r) FHR-1, FHR-2 and FHR-5, produced in HEK293F cells and obtained monoclonal antibodies that were screened for binding specificity and cross-reactivity against rFHRs and plasma-derived FH. FHR dimerization was investigated using pull-down assays, sucrose gradients and heparin chromatography. rFHR-2 and FHR-5 were fluorescently labeled to study monomer exchange via FRET assays.

We demonstrate that FHR-1 and FHR-2 can exchange monomers and are equally able to form homo- and heterodimers, whereas FHR-5 can form only homodimers. The kinetics indicate that the dimerization and monomer exchange is a fast process and free monomers presumably do not exist. Sucrose gradients of serum from healthy donors confirmed that serum-derived proteins circulate as dimers *in vivo* as well. Also subsequent immunoprecipitations of FHR-1 or FHR-2 from human serum demonstrated that FHR-1 and FHR-2 are present as both homo- and heterodimers. These dimer species all have their own heparin binding capacity. FHR-1 homodimers bind heparin at a similar strength as FH, while FHR-2 homodimers and FHR-1/FHR-2 heterodimers have minimal and intermediate heparin binding capacity, respectively.

This research advances our knowledge about the factor H-related proteins. The now elucidated dimer profile allows for accurate measurements of FHR plasma levels and provides new insights on the role of these dimers in complement regulation.

Taco W. Kuijpers and Diana Wouters contributed equally to this work.

http://dx.doi.org/10.1016/j.imbio.2016.06.130

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A functional role for complement receptor C5L2 in the pathogenesis of renal ischemia-reperfusion injury

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The complement system, and specifically C5a, is involved in renal ischemia-reperfusion (IR) injury. The two receptors for C5a, C5aR and C5L2, are expressed on leukocytes as well as in the kidney. Extensive evidence shows that C5aR inhibition protects kidneys from IR injury, but the role of C5L2 in IR injury has not been studied so far. Therefore, WT, C5aR−/− and C5L2−/− mice were subjected to 40 min of bilateral renal ischemia, followed by reperfusion for 1, 3 or 7 days. It was found that C5L2−/− mice were protected against IR injury, resulting in significant lower plasma creatinine and BUN levels, and reduced acute tubular necrosis. Next, an in vivo migration study, where WT, C5aR−/− and C5L2−/− mice were injected intraperitoneally with complement ligands, revealed that C5L2 is not involved in leukocyte migration. To investigate the contribution of renal-expressed C5L2 versus leukocyte-expressed C5L2 to renal IR injury, bone marrow chimeras were created. Our data show that renal-expressed C5L2 and leukocyte-expressed C5L2 mediate IR injury-induced renal dysfunction. Therefore, C5L2 is a functional receptor in renal IR injury rather than a simple decoy receptor. For that reason, next to C5aR, C5L2 is a potential target for intervention during renal IR injury.

http://dx.doi.org/10.1016/j.imbio.2016.06.131

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Low mannose-binding lectin levels predict cardiovascular disease in hemodialysis patients

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Background: Hemodialysis (HD) is a lifesaving treatment for patients with end stage renal disease not suitable for renal transplantation or awaiting a donor kidney. However, patients receiving HD have much higher rates of cardiovascular morbidity and mortality compared to the general population. Mannose-binding lectin (MBL) has been shown to play an important role in the development of cardiovascular disease. However, this relationship is complex and studies suggest that MBL can be either detrimental or beneficial. In addition, it has been shown that MBL concentration and functionality are altered by HD. Therefore, this study aimed to determine the predictive value of MBL levels for cardiac and cardiovascular events and all-cause mortality in HD patients.

Methods: We conducted a prospective study of 107 HD patients on maintenance dialysis. Plasma MBL was measured before and after HD using a sandwich ELISA. The prognostic effect of MBL levels after HD was evaluated using Cox regression models. The primary endpoint was the incidence of cardiac (C-event) and cardiovascular events (CV-event). The secondary endpoint was all-cause mortality.

Results: During median follow-up of 27 months, 21 participants (20%) developed C-events, while 36 (34%) had CV-events. Furthermore, 37 patients (35%) died during the study and 21 (20%) received a kidney transplant. After the study period, 58% of patients with low MBL levels (<319 ng/mL) and 26% of patients with high MBL levels (≥319 ng/mL) had a CV-event. The incidence of both cardiac and cardiovascular events was significantly higher in patients with low MBL levels. Low MBL levels were associated with a hazard ratio of 2.64 (95% CI, 1.36–5.13; $p = 0.004$) for a CV-event and 2.60 (95% CI, 1.10–6.18; $p = 0.03$) for a C-event. After adjustment, the hazard ratio for future CV-event was 3.98 (95% CI, 1.88–8.24; $p < 0.001$) or 3.96 (95% CI, 1.49–10.54; $p = 0.006$) for C-event in HD patients with low MBL levels. No association was found between low MBL levels and all-cause mortality.

Conclusion: Low MBL is associated with a higher risk of cardiac and cardiovascular events, but not all-cause mortality. Therefore,

MBL levels may help to identify HD patients who are susceptible to develop cardiovascular disease.

http://dx.doi.org/10.1016/j.imbio.2016.06.132

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Protective role of PEG conjugated phospholipid in reducing ischemic reperfusion injury in two allogeneic pig kidney transplant models

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Background: Ischemia/reperfusion (I/R) is a potent inducer of complement and coagulation activation (thromboinflammation), cytokines and inflammatory mediator generation leading to organ damage during transplantation.

Previously we have utilized a PEG-lipid construct that spontaneously inserts into cell membranes. The PEG portion can be functionalized with various ligands. We successfully regulated thromboinflammation induced in a xenogeneic model in which PAECs came into direct contact with human blood by conjugating a combination of apyrase to cleave ADP and a factor H-specific peptide to bind factor H to the surface, thereby inhibiting both platelet and complement activation.

Aim: We wanted to explore whether the native, non-functionalized PEG-lipid *per se* has a protective effect in I/R. In order to address this issue we used two allo-geneic kidney transplant models in pigs, which enables a perfect matched control for each treated kidney.

Methods: In a short-term acute model, the two kidneys from one pig were procured *en bloc* and stored for 24 h at 4 °C in HTK solution. One hour before transplantation, the kidneys were treated with the PEG-lipid or buffer (control kidney). Thereafter, the two *en-bloc* kidneys were transplanted into an allogeneic pig, washed out *in situ*, and then the kidneys were followed for 6 h.

In a long-term survival model, the kidneys of a single donor pig were removed and stored for 24 h at 4 °C. Before transplantation, one of the kidneys was treated with the PEG-lipid and the other with buffer for 1 h; after washout, the kidneys were transplanted into two additional SLA-matched sibling pigs, whose kidneys had been removed. The animals were not immunosuppressed, and they were followed for 4–5 days.

Results: A profound difference was observed in both models. Compared to the controls, the initial deposition of complement C3b in the parenchyma, coagulation and complement activation in plasma (TAT, C3a, sC5b-9), and IL-1beta and IL-6 were depressed in the treated animals. In the long-term model, creatinine was also significantly lower in the treated animals.

Significance: These experiments show that the new technique with PEG-lipid coatings is very successful in lowering the innate immune response during allogeneic transplantation in large animal models. Further development of new specific regulators has the potential to further improve the technology.

http://dx.doi.org/10.1016/j.imbio.2016.06.133

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C3 gene polymorphism and cardiometabolic risk factors in chronic Chagas disease

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Chagas disease (CD) is a chronic, parasitic infection caused by *Trypanosoma cruzi*, which affects about 8 million people in Latin America and is an emerging problem worldwide due to international migration. Although most individuals infected by *T. cruzi* remain asymptomatic in the indeterminate phase lifelong, 30–40% of them will progress to symptomatic disease, being cardiomyopathy (CCC) 20–30%, digestive megasyndromes 15–20, or both up to 10%. The complement system plays an important role in reducing parasite load during acute *T. cruzi* infection, however, complement activation can also cause tissue injury due to inflammation in CCC and contributing therefore to the clinical progression of chronic CD. Considering the central role of C3 in the activation of complement system we aimed to investigate the impact of *C3* gene on the susceptibility and clinical progression to CD. We investigate whether functional cardiac parameters and *C3* gene polymorphisms could be useful markers in the progression of indeterminate to chronic symptomatic CD. *C3* gene polymorphisms (rs2250656T > C, rs2230199C > G: Arg80Gly and rs1047286G > A: Pro292Leu) were genotyped in 123 chronic CD patients (78 indeterminate, 29 cardiac and 16 cardiodigestive forms) and 148 controls from Southern Brazil, using sequence-specific PCR. Clinical evaluation was performed in two different periods (2005 and 2015) with lipidogram, echocardiography (ECHO) and electrocardiogram assessments in the chronic CD patients. *C3 TGG* haplotype presented a protective effect against the development of CD ($p = 0.02$ and OR 0.13). On the other hand, the *C3 TCA* haplotype was associated with the susceptibility of indeterminate CD when compared to controls ($p = 0.004$ and OR 5.48) and showed a protective effect against the disease progression to symptomatic CD ($p = 0.041$ and OR 0.08). During the eleven years of follow-up, (2005–2015), 28.2% of the indeterminate patients progressed to symptomatic forms. None of the progressors carried the *TCA* haplotype. In addition, Left Ventricle End-Diastolic Diameter (LVED) assessed by ECHO in 2005 was significantly higher in the patients that progressed when compared to non-progressors ($p = 0.0015$ and RR 3.7, when LVED $\geq$ 50 mm). No significative difference was observed for all the other parameters. In conclusion, *C3* gene polymorphisms and LVED may be potential markers to indicate the progression from indeterminate to chronic symptomatic CD.

http://dx.doi.org/10.1016/j.imbio.2016.06.134