An analysis of functional activity via the three complement pathways during hemodialysis sessions: a new insight into the association between the lectin pathway and C5 activation

Hiroyuki Inoshita, Isao Ohsawa, Kisara Onda, Mariko Tamano, Satoshi Horikoshi, Hiroyuki Ohi and Yasuhiko Tomino

1Division of Nephrology, Department of Internal Medicine, Faculty of Medicine, Juntendo University, Bunkyo-ku, Tokyo, Japan, 2Department of Pathology, Case Western Reserve University, Cleveland, OH, USA and 3Tsurumi-Nishiguchi Hospital, Yokohama, Kanagawa, Japan

Correspondence and offprint requests to: Yasuhiko Tomino; E-mail: yasu@juntendo.ac.jp

Abstract

Background. We have recently demonstrated that hemodialysis (HD) patients have significantly higher levels of functional complement activity (FCA) in all three pathways, i.e. the classical pathway, alternative pathway and lectin pathway (LP), than in age-matched controls, though the role of FCA during HD still remains unknown.

Methods. Serial plasma or serum samples were obtained from five patients during HD in order to investigate the kinetics of complement components. The levels of the C5b-9 complex, the FCA of the three pathways, a derivative of C3a (C3a desArg) and a derivative of C5a (C5a desArg) in the samples were analyzed.

Results. The levels of the C5b-9 complex at 60 min were significantly increased when compared with those at 0 min. Functional activities for all three pathways showed different patterns so the same tendency between pathways was not observed. The levels of C3a desArg and C5a desArg at 60 min were markedly increased when compared with those at 0 min. A Spearman’s rho test showed a strong positive correlation between functional LP activity and C5a desArg.

Conclusions. These findings lead to new insights into the FCA during HD and suggest that functional LP activity has an important role in C5 activation.

Keywords: complement; C3a desArg; C5a desArg; hemodialysis; lectin pathway

Introduction

Under normal physiological conditions, complement activation leads to a proteolytic cascade resulting in immune cell activation, rapid opsonization and the elimination of microorganisms. However, excessive complement activation, especially in the generation of the C3a, C5a and C5b-9 complexes, are life-threatening for patients on hemodialysis (HD) [1–6]. The C3a, C5a and C5b-9 complexes are generated from the activation of C3 and C5 via three complement activation pathways, the classical pathway (CP), alternative pathway (AP) and lectin pathway (LP). Therefore, although little is known about it, clarifying the activation process in the three pathways during HD sessions is of clinical importance.

Recent advances allow us to assess the functional complement activity (FCA) of the three pathways independently, and in parallel, by using the novel ELISA (Wielisa®-kit) instead of a hemolytic assay. Our recent study using this method has demonstrated that HD patients had significantly higher levels of FCA in all three pathways than in age-matched controls [7], although the role of the fluctuations in the FCA during HD is still unknown. Although measurements of C3a and C5a have been used as a monitoring parameter of complement activation during HD, C3a and C5a are immediately digested by carboxypeptidase N and processed to their more stable metabolites C3a desArg and C5a desArg in vivo [8, 9]. Thus, the measurement of C3a desArg and C5a desArg should reflect the physiological condition of C3a and C5a more accurately than by just measuring C3a and C5a.

In the present study, we first revealed the kinetic changes in the FCA of the three complement pathways during HD and also assessed the association between the three activation pathways and C3a desArg and C5a desArg.

Materials and methods

Patients and study design

The characteristics of the enrolled patients are listed in Table 1. In this study, two different HD membranes were...
used: a cellulose membrane (CL-EE®, Asahi Medical Co. Ltd., Tokyo, Japan) (n = 3) and a polysulfone membrane (APS®, Asahi Medical Co. Ltd.) (n = 2). Because patient No. 3 had residual renal function, the \( Kt/V \) value was lower than in other patients. None of the patients manifested any infection or malignancy symptoms. Patients with a history of severe infection, unstable erythropoietin dosage or single-needle dialysis were excluded from this study. All of the patients gave their informed consent to participate in this study, which was performed in compliance with the Helsinki Declaration.

Samples

Samples were obtained from the arterial side of the arteriovenous fistula before an anticoagulant injection (0 min). Subsequently, the samples were serially obtained from the arterial line of the dialyzer. After centrifugation, these samples were stored at \(-80^\circ\text{C}\) prior to the processing.

Measurements

The measurement of FCA was performed using a Wielisa®-kit (Wieslab, Lund, Sweden) as described previously [7]. In brief, the wells of the microtiter strips were coated with specific activators for the classical, alternative or lectin pathways. Patient serum was diluted with different specific blockers to ensure that only a specific pathway was activated [10]. After activation, the C5b-9 complex was captured using an alkaline phosphatase-conjugated antibody, after which color development was measured (Figure 1A). The C5b-9 complex rapidly increased until 60 min and plateaued until the end of HD (100.0 ± 9.608, 291.8 ± 43.41, 414.5 ± 28.66, 313.3 ± 78.72, 344.3 ± 41.73), with the mean levels at 60 min significantly higher than those at 0 min (\(P = 0.0037\)). This result led us to focus on the early phase, up until 60 min, during HD. There was the same tendency in the total levels of the C5b-9 complex between HD patients who used a cellulosic membrane and those who used a polysulfone membrane (data not shown). To examine the kinetics change of the FCA in the three complement pathways during HD, the activity of each was measured in the serum samples at 0, 15, 30 and 60 min (Figure 1B). FCA via the CP was lower at 15 min and then increased as time passed (97.43 ± 28.07, 90.99 ± 21.29, 95.03 ± 33.43, 104.8 ± 24.96). FCA via the AP was lower at 15 min and reached a plateau until 60 min (95.21 ± 26.21, 83.37 ± 25.42, 82.82 ± 30.37, 83.15 ± 31.69). FCA via the LP was slightly higher at 15 min and slightly lower at 60 min (106.7 ± 24.74, 115.3 ± 21.65, 114.6 ± 28.79, 111.7 ± 30.04). There were no significant differences in any of the three pathways when comparison was made between 0 and 60 min. The same tendency was not observed in FCA in the three complement pathways. To evaluate the C3 and C5 activity during HD, the levels of C3a desArg and C5a desArg in the samples at 0, 15, 30 and 60 min were measured (Figure 1C). Both C3a desArg and C5a desArg tended to be rapidly increased until 15 min, and then reached a plateau (C3a desArg; 101.1 ± 5.519, 115.0 ± 5.152, 112.9 ± 4.62, 115.4 ± 6.612, C5a desArg; 106.3 ± 11.97, 128.6 ± 13.32, 127.8 ± 10.92, 133.8 ± 16.96). Especially, C3a desArg at 60 min was significantly higher than at 0 min (\(P = 0.047\)). Finally, we assessed the correlation between the FCA of three pathways and the levels of C3a desArg and C5a desArg in the total of 20 serum samples that were collected from these five patients at all points (0, 15, 30 and 60 min) in the early phase of HD. The analysis showed a strong positive correlation between only the FCA via LP activity and C5a desArg (Table 2 and Figure 2).

### Discussion

Our previous studies suggest that the complement system has important roles in HD patients [4, 7, 11–13]. This is the first study investigating the kinetics of the FCA of the three complement pathways simultaneously during HD. Total levels of the C5b-9 complex at 60 min were significantly higher than those at 0 min, and the three FCAs each showed a different pattern. These results suggest that the total levels of the C5b-9 complex may serve as a good marker for the evaluation of
biocompatibility and that the FCA of the three complement pathways occurs independently during HD.

This study also showed that the FCA via the LP is correlated with the levels of C5a desArg, which is the more stable metabolite of C5a during HD. The result suggests that the LP is the main contributor in the generation of C5a. The LP, the most recently discovered of the three complement pathways, is triggered through recognition of mannose-binding lectin, L-ficolin and H-ficolin to carbohydrate [14, 15]. The activated LP results in activated C3, which acts as a C5 convertase. Eventually, the C5b-9 complex is formed through the terminal pathway activated by the cleavage of C5. The cleavage of C5 generates C5a, which not only contributes to the formation

Fig. 1. The kinetics of the complement system in the early phase of HD. (A) The levels of the C5b-9 complex in plasma samples obtained from patients during HD at 0, 20, 60, 180 min and post-HD. Statistical significance was assessed between 0 and 60 min. ***P < 0.005. (B) The three functional complement pathway activities in serum samples obtained from patients during HD at 0, 15, 30 and 60 min. Statistical significance was assessed between 0 and 60 min. (C) The levels of C3a desArg and C5a desArg in serum samples were obtained from patients during HD at 0, 15, 30 and 60 min. *P < 0.05. Statistical significance was assessed between 0 and 60 min. All data are shown as the mean ± standard error. n = 5 for each group.
of the C5b-9 complex, leading to cell damage, but it also activates macrophages, helper T cells and B cells. Thus, the action of C5a results in the release of numerous proinflammatory cytokines and chemokines, such as IL-6, IL-8 and tumor necrosis factor [16], and therefore, the blocking of C5 activation is the key to anti-inflammatory treatment. Recently, Mares et al. [17, 18] analyzed proteins adsorbed to dialyzers by two-dimensional electrophoresis and the levels of complement components involved in the LP in plasma samples from patients during HD. They showed that enriched L-ficolin was observed in the eluates of dialyzers and that there is a strong association between L-ficolin and C5a in the early phase of HD, suggesting that L-ficolin adsorption to the dialyzer initiates the LP of complement activation. Our future studies should focus on the mechanism of L-ficolin activation by binding to the dialyzer in the early phase of HD.

In conclusion, our results could help in the understanding of the FCA during HD and suggest that the LP is a potential target in avoiding the generation of C5a and C5b-9 complexes.

Acknowledgements. We would like to express our gratitude to all those who work in Kasukabe Kisen Hospital for giving us the opportunity to use their clinical data to complete this thesis.

Conflict of interest statement. None declared.

References

1. Cheung AK, Parker CJ, Wilcox LA et al. Activation of complement by hemodialysis membranes: polyacrylonitrile binds more C3a than cuprophan. Kidney Int 1990; 37: 1055–1059

2. Cheung AK, Faezi-Jenkin B, Leypoldt JK. Effect of thrombosis on complement activation and neutrophil degranulation during in vitro hemodialysis. J Am Soc Nephrol 1994; 5: 110–115

3. Cheung AK, Parker CJ, Hohnholt M. Soluble complement receptor type 1 inhibits complement activation induced by hemodialysis membranes in vitro. Kidney Int 1994; 46: 1680–1687

4. Tamano M, Ohi H, Sudo S et al. Quantitative polymorphism of complement receptor type 1 (CR1) in patients undergoing haemodialysis. Nephrol Dial Transplant 2004; 19: 1467–1473

5. Vaisar T, Pennathur S, Green PS et al. Shotgun proteomics implicates protease inhibition and complement activation in the anti-inflammatory properties of HDL. J Clin Invest 2007; 117: 746–756

6. Kourtzelis I, Markiewski MM, Doumas M et al. Complement anaphylatoxin C5a contributes to hemodialysis-associated thrombosis. Blood 2010; 116: 631–639

7. Inoshita H, Ohsawa I, Kasaba G et al. Complement in patients receiving maintenance hemodialysis: functional screening and quantitative analysis. BMC Nephrol 2010; 11: 34

8. Huey R, Bloor CM, Kawahara MS et al. Potentiation of the anaphylatoxins in vivo using an inhibitor of serum carboxypeptidase N (SCPN). I. Lethality and pathologic effects on pulmonary tissue. Am J Pathol 1983; 112: 48–60

9. Kretzler DL, McCormick JR, Despins A et al. Characterization of the anaphylatoxin inactivator and chemotactic factor inactivator activities during cardiopulmonary bypass. J Exp Pathol 1984; 1: 183–187

10. Inoshita H, Matsushita M, Koide S et al. A novel measurement method for activation of the lectin complement pathway via both mannose-binding lectin (MBL) and L-ficolin. J Immunol Methods 2009; 349: 9–17

11. Wakabayashi H, Ohi H, Tamano M et al. Acquired loss of erythrocyte complement receptor type 1 in patients with diabetic nephropathy undergoing hemodialysis. Nephron Exp Nephrol 2006; 104: e89–e95

12. Ohsawa I, Ohi H, Maruyama T et al. Leukocytapheresis (LCAP) for the treatment of rheumatoid arthritis on maintenance hemodialysis patient. Clin Nephrol 2007; 68: 121–124

13. Ishii M, Ohsawa I, Inoshita H et al. Serum concentration of complement components of the lectin pathway in maintenance hemodialysis patients, and relatively higher levels of L-ficolin and MASP-2 in mannsos-binding lectin deficiency. Ther Apher Dial 2011; 15: 441–447

14. Matsushita M, Endo Y, Taira S et al. A novel human serum lectin with collagen- and fibrinogen-like domains that functions as an opsonin. J Biol Chem 1996; 271: 2448–2454

15. Matsushita M, Endo Y, Fujito T. Cutting edge: complement-activating complex of ficolin and mannose-binding lectin-associated serine protease. J Immunol 2000; 164: 2281–2284

16. Zhang X, Kimura Y, Fang C et al. Regulation of Toll-like receptor-mediated inflammatory response by complement in vivo. Blood 2007; 110: 228–236

17. Mares J, Thongboonkerd V, Tuma Z et al. Specific adsorption of some complement activation proteins to polysulfone dialysis membranes during hemodialysis. Kidney Int 2009; 76: 404–413

18. Mares J, Richtrova P, Hricinova A et al. Proteomic profiling of blood-dialyzer interactome reveals involvement of lectin complement pathway in hemodialysis-induced inflammatory response. Proteomics Clin Appl. 2010; 4: 829–838

Received for publication: 26.4.12; Accepted in revised form: 29.6.2020