HMGB1 release by C5a anaphylatoxin is an effective target for sepsis treatment

Noriko Okada*, Masaki Imai*, Alan Okada[&], Fumiko Ono[#] and Hidechika Okada*[,&]

*Department of Immunology, Nagoya City University Graduate School of Medical Sciences, Nagoya 467-8601, [&]Choju Medical Institute, Fukushimura Hospital, Toyohashi 411-8124, and [#]Tsukuba Primate Research Center, National Institute of Biomedical Innovation, Tsukuba 305-0843, Japan

Address correspondence and reprint request to Dr. Hidechika Okada, Choju Medical Institute, Fukushimura Hospital, Noyori-cho, Yamanaka 19-14, Toyohashi 441-8124, Japan E-mail: hiokada@med.nagoya-cu.ac.jp

Key words: complement; C5a; anaphylatoxin; inflammation; sepsis; peptide; endotoxin-shock; C5a receptor; C5L2; HMGB1; cytokine storm.

Antibodies to C5a have proven to be effective in treating experimental septic primate models[1,2]. A 17 amino acid peptide (ASGAPAPGPAGPLRPMF) named PepA binds to C5a and prevents complement-mediated lethal shock in rats[3]. AcPepA harboring an acetyl group at the N-terminal alanine showed increased inhibitory activity against C5a[4]. Cynomolgus monkeys destined to expire from a lethal dose of bacterial endotoxin (4mg/kg) were rescued by intravenous administration of AcPepA. AcPepA could have interfered with the ability of C5a to stimulate C5L2[5,6] which is responsible for HMGB1 release and stimulation of TLR4[7-9] as an endogeneous ligand with LPS behavior. The suppression of HMGB1 release by AcPepA administration to LPS-shock monkeys is likely responsible for rescuing the animals.

Sepsis is a systemic inflammatory response syndrome (SIRS) that causes disseminated intravascular coagulation (DIC) and multiple organ failure (MOF). Antibodies to C5a have proven to be effective in treating experimental septic primate models[1,2]. We generated an inhibitory peptide of C5a composed of an amino acid sequence ASGAPAPGPAGPLRPMF named PepA[3] . Acetylation at the N-terminal alanine of PepA improved the C5a inhibitory capacity and was named AcPepA[4].

Under anesthesia with sodium pentobarbital, 10 cynomolgus monkeys (weighing about 5 kg) were intravenously administered 4 mg/kg LPS within 30 min. Three monkeys for the control group were infused with 15 ml saline during 3 hrs after the LPS injection. Seven experimental group monkeys were infused intravenously with 15 ml of 2 mg/ml AcPepA starting at 30 min after LPS injection for 3 hrs (2 mg/kg/hr for 3 hrs). Six hrs after LPS administration, anesthesia was terminated when the blood samples showed leukocytosis and increased CPK in all monkeys. Monkeys were observed for their status. All of the 7 AcPepA treated monkeys returned to a healthy condition by the following day, while the 3 control monkeys died within two days.

Despite the increased TNFα and other cytokine levels, high mobility group box 1 (HMGB1)[5,6] which is an endogenous stimulator of TLR4[7-9] did not increase in the AcPepA infused animals (Fig. 1).

Furthermore, AcPepA could suppress pathophysiological events and prolonged survival time of sepsis piglets induced by cecal ligation and perforation (CLP)[10]. Survival times were longer in the AcPepA treated group than in the CLP alone group (19.3hrs ± 2.7hrs vs. 9.9 hrs ± 0.7 hrs, $P<0.005$). In this case, AcPepA also delayed the HMGB-1 surge.

These above results indicate that suppression of C5 anaphylatoxin interferes with

the induction of a cytokine storm. Since C5a has the capacity to cause release of HMGB1 following stimulation of the second C5a receptor termed C5L2 generated on activated monocytes[11-13], inhibition of C5a successfully interferes with the above release which has the capacity to generate inflammatory cytokines stimulating TLR4 as an endogenous ligand (Fig. 2).

Recently, thrombomodulin (TM) administration has been shown to rescue septic shock animals[14]. The enhanced activity of thrombin when complexed with TM should have caused activation of thrombin activatable fibrinolysis inhibitor (TAFI) which then inactivates C5a anaphylatoxin by removing the C-terminal arginine[15,16] resulting in suppression of HMGB1 release. Therefore, the therapeutic effect of TM on sepsis should also be due to inactivation of C5a anaphylatoxin which initiates a cytokine storm through HMGB1 release.

This work was supported in part by a Research Grant from the Japanese Ministry of Health, Welfare and Labor (08062893).

References

1. Czermak, B.J., V. Sarma, C. L. Pierson, R. L. Warner, M. Huber-Lang, N. M. Bless, H. Schmal, H. P. Friedl, and P. A. Ward, 1999. Protective effects of C5a blockade in sepsis. *Nature.Med.* **5:** 788-792.

2. Stevens, J.H., P. O'Hanley, J.M. Shapiro, F.G. Mihm, P.S. Satoh, J.A. Collins, and T.A. Raffin. 1986. Effects of anti-C5a antibodies on the adult respiratory distress syndrome in septic primates. *J. Clin. Invest.* **77**: 1812-1816.

3. Fujita, E., I. Farkas, W. Campbell, L. Baranyi, H. Okada, and N. Okada. 2004. Inactivation of C5a anaphylatoxin by a peptide that is complementary to a region of C5a. *J. Immunol.* **172**: 6382-6387.

4. Okada, N., S. Asai, A. Hotta, N. Miura, N. Ohno, I. Farkas, L. Hau, and H. Okada. 2007. Increased inhibitory capacity of an anti-C5a complementary peptide following acetylation of N-terminal alanine. *Microbiol. Immunol.* **51**: 439-443.

5. Wang, H., O. Bloom, M. Zhang, J.M. Vishnubhakat, M. Ombrellino, J. Che, A. ., Frazier, H. Yang, S. Ivanova, L. Borovikova, K.R. Manogue, E. Faist, E. Abraham, J. Andersson, U. Andersson, P.E. Molina, N.N. Abumrad, A. Sama, and K.J. Tracey. 1999. HMGB-1 as a late mediator of endotoxin lethality in mice. *Science* **285**: 248-251.

6. Riedemann NC, R-F. Guo RF, and P.A. Ward. 2003. Novel strategies for the treatment of sepsis. *Nature. Med.* **9**: 517-524.

7. Yu, M., H. Wang, A. Ding, D.T. Golenbock, E. Latz, C.J. Czura, M.J. Fenton, K.J. Tracey, H. Yang. 2006. HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. *Shock* **26**: 174-179.

8. Yang, H., M. Ochani, J. Li, X. Qiang, M. Tanovic, H. Harris, S.M. Susaria, M.P. Ulloa, H. Wang, R. DiRimo, C.J. Czura, H.Wang, J. Roth, H.S. Warren, H.S., M.P. Fink, M.J. Fenton, U. Andersson, and K.J. Tracey. 2004. Reversing established sepsis with antagonists of endogeneous high-mobility group box 1. *Proc. Natl. Acad. Sci.* **101**: 296-301.

9. Park, J.S., F. Gamboni-Robertson, Q. He, D. Svetkauskaite, J.Y. Kim, D. Strassheim, J.W. Sohn, S. Yamada, I. Maruyama, A. Banerjee, A. Ishizaka, and E. Abraham, E. 2006. High mobility group box 1 protein interacts with multiple Toll-like receptors. *Am. J. Physiol. Cell. Physiol.* **290**: C917-924.

10. Hussein, MH., Kato, S., Goto, T., Daoud, GA., Kato, I., Suzuki, S., Togari, H., Imai, M., Okada, N., Okada, H. An acetylated anti-C5a complementary peptide reduced cytokines and free radicals and prolongs survival time in a neonatal sepsis model **Mol Immunol** 2009, 46:2825

11. Chen, N-J., Mirtsos, C., Suh, D., Lu, Y-C., Lin, W-J., McKerlie, C., Lee, T., Baribault, H., Tian, H. and Yeh, W-C. 2007. C5L2 is critical for the biological activities of the anaphylatoxins C5a and C3a. *Nature* 446: 203-207.

12. Klune, J.R., Dhupar, R., Cardinal, J., Billiar, T.R. and Tsung, A. 2008. HMGB1:endogeneous danger signaling. Mol. Med. 14:476-484.

13. Rittirsch, D., Flierl, M.A., Nadeau, B.A., Day, D.E., Huber-Lang, M., Mackay, C.R., Zetoune, F.S., Gerard, N.P., Cianflone, K., Koehl, J., Gerard, C., Sarma, J.V. and Ward, P.A. 2008. Functional roles for C5a receptors in sepsis. *Nature Med.* **14**: 551-557.

14. Nagato M, Okamoto K, Abe Y, Higure A, Yamaguchi K. 2009. Recombinant human soluble thrombomodulin decreases the plasma high-mobility group box-1 protein

levels, whereas improving the acute liver injury and survival rates in experimental endotoxemia. *Crit Care Med.* **37:**:2181-2186.

15. Campbell, W., N. Okada, and H. Okada. 2001. Carboxypeptidase R (CPR) is an inactivator of complement derived inflammatory peptides and an inhibitor of fibrinolysis. *Immunol. Rev*. **180:** 162-167.

16. Campbell, W., E. Lazoura, N. Okada, and H. Okada. 2002. Inactivation of C3a and C5a octapeptides by carboxypeptidase R and carboxypeptidase N. *Microbiol. Immunol*. **46:**131-134.

(Fig. 1)

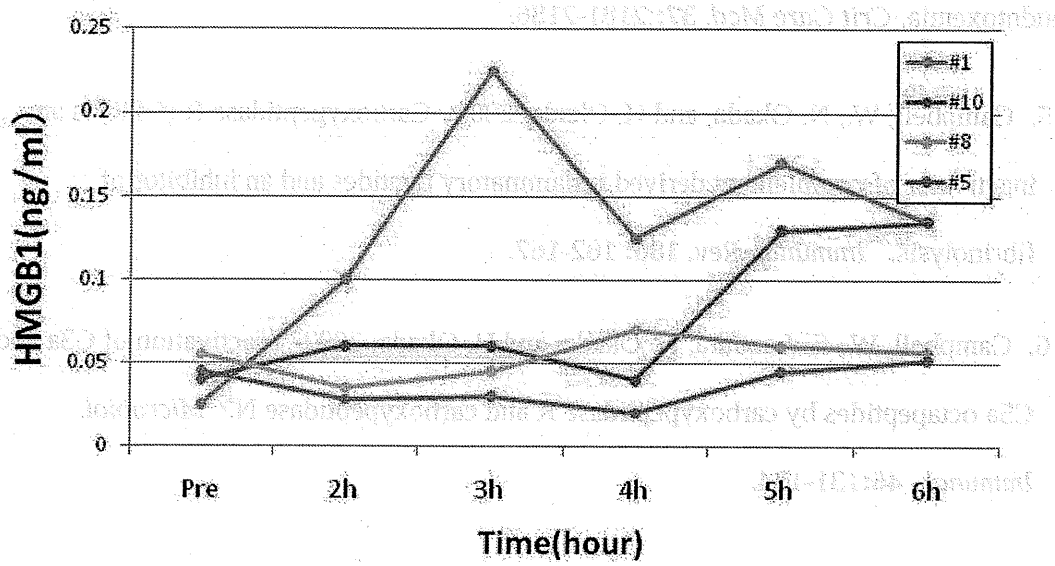

Figure 1. Increase in HMGB1 in plasma of LPS- injected monkeys.

Six cynomolgus monkeys intravenously infused with a lethal dose of bacterial LPS (4mg/kg) destined to death were treated with intravenous administration of 2 mg/kg/h of AcPepA for 3h starting 30 min after the lethal LPS injection (#5 and #8).. Control monkeys (#1 and #10) were infused only saline in stead of AcPepA following LPS injection. Despite the increased TNFα and other cytokine levels, high mobility group box 1 (HMGB1) which is an endogenous stimulator of TLR4[7] did not increase in the AcPepA infused animals (#5 and #8).

(Fig. 2)

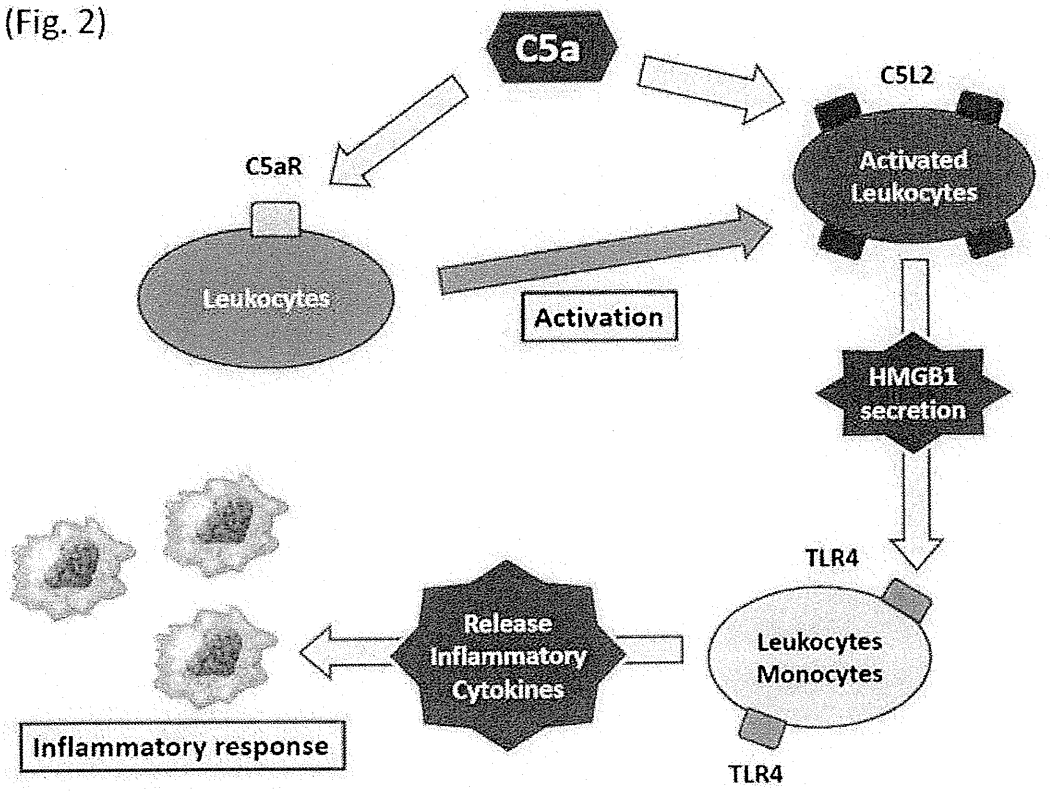

Figure 2. Possible role for C5a in a positive feedback inflammatory circuit.

Following bacterial infection, LPS stimulates TLR4, and C5a generated during complement activation stimulates C5aR resulting in expression of C5L2 on leukocyte membranes. Stimulation of C5L2 by C5a on activated leukocytes induces release of HMGB1 which then reacts with TLR-4 on other leukocytes, as did LPS, resulting in further recruitment of activated leukocytes that express C5L2. These reactions create an inflammatory amplification circuit.

厚生労働省　創薬基盤推進研究事業

黄斑変性カニクイザルを用いた補体抑制による加齢黄斑変性の予防薬の開発

(H21-創薬一般-002)

平成23年度　総括研究報告書

岩田　岳

平成24年5月