Novel Complementary Peptides to Target Molecules

HIDECHIKA OKADA[1], MASAKI IMAI[2], FUMIKO ONO[3], ALAN OKADA[1], TOYOHIRO TADA[4], YUKA MIZUE[5], KEIJI TERAO[3] and NORIKO OKADA[2]

[1]*Research Institute for Protein Science, Nakayama-cho 2-18, Mizuho-ku, Nagoya 467-0803, Japan;*
[2]*Nagoya City University Graduate School of Medical Sciences, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan;*
[3]*Tsukuba Primate Research Center, National Institute of Biomedical Innovation, Tsukuba 305-0843, Japan;*
[4]*Nagoya City University School of Nursing, Nagoya 467-8601, Japan;*
[5]*Sapporo Immuno Diagnostic Laboratory, Sapporo 001-0922, Japan*

Abstract. *We generated an evolutionary computer program that generates complementary peptide (C-pep) sequences, with the potential to interact with a target peptide, by comparing several physico-chemical parameters of each pair of the complementary peptides being analyzed. We generated C-peps to target several molecules. About 30% of synthesized C-peps interfered with the function of their targets. C5a stimulates generation of TNFα and other inflammatory cytokines. Inhibition of C5a should be effective against sepsis, which impairs the status of cancer-bearing patients. One of the inhibitory C-peps of C5a, termed AcPepA, was effective in Cynomolgus monkeys intravenously infused with a lethal dose of bacterial LPS (4 mg/kg) destined to die. The monkeys were rescued by intravenous administration of 2 mg/kg/h of AcPepA. The excellent therapeutic effect of AcPepA is likely to be due to restriction of high mobility group box 1 (HMGB1) surge induced by the effect of C5a on C5L2, which is the second C5a receptor, since the released HMGB1 has the capacity to stimulate TLR4 as an endogeneous ligand resulting in further activation of inflammatory cells to release inflammatory cytokines forming a positive feedback circuit of inflammation.*

After proposal of the possible role of antisense peptides for molecular interaction among proteins by Blalock *et al*. (1) in 1984, the theory was reviewed later (2, 3). Many examples of sense–peptide and antisense–peptide relationships have been found between receptors and their protein ligands (4-8). We speculated that such interactions between sense and antisense peptides should play a role in formation of the tertiary structure of proteins. We developed a novel computer program named ANTIS to find antisense peptide sequences between proteins to be compared (9). By analyzing intramolecular antisense peptides within a single protein molecule, we found that there are an appreciable number of sense and antisense peptide pairs within a protein molecule and we designated these as antisense homology boxes (AHB) (9). Using ANTIS, we analyzed sense and antisense peptide relationships in the endothelin receptor (ETR) molecule and between endothelin and ETR. One of the AHB peptides of ETR, named ETR-P1/fl, had the capacity to interfere with the function of ETR (10). We expected that it would be possible to generate candidates of complementary peptide reactive to a target amino acid sequence based upon the sense-antisense amino acid relationship. We generated an evolutionary computer program that runs on any PC, and generates complementary peptide (C-pep) sequences, with the potential to interact with a target peptide, by comparing several physico-chemical parameters of each pair of the complementary peptides being analyzed (11). With the program named MIMETIC, we generated complementary peptides (C-peps) to HIV-reverse transcriptase (11, 12), procarboxypeptidase R, thrombomodulin (13), and C5a anaphylatoxin (14) as listed in Table I. About 30% of the synthesized peptides interfered with the function of their target molecules. Out of 19 complementary peptides (C-peps) targeted to C5a anaphylatoxin, 7 exhibited an inhibitory effect.

C5a is a 74 amino acid peptide released from the fifth component of complement (C5) by C5 convertase generated during complement activation (15). C5a anaphylatoxin is considered to be an effective target for treatment of hyperinflammation since C5a stimulates generation of tumor necrosis factor alpha (TNFα) and other inflammatory cytokines (16-18). Although C5a generated *in vivo* is regulated by carboxypeptidase N and more efficiently by carboxypeptidase R (CPR) (19, 20), excessive generation of

Correspondence to: H. Okada, Research Institute for Protein Science, Nakayama-cho 2-18, Mizuho-ku, Nagoya 467-0803, Japan. Tel: +81 9027738012, Fax: +81 528535112, e-mail: hiokada@med.nagoya-cu.ac.jp

Key Words: Antisense pepitide, complementary peptide, anaphylatoxin, inflammation, sepsis, cytokine storm.

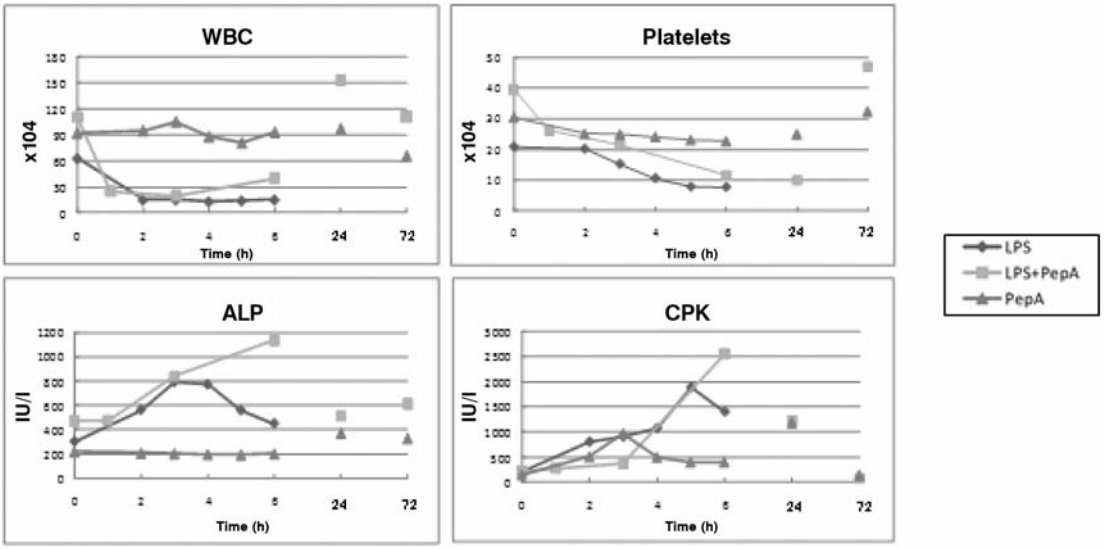

Figure 1. *Clinical features in peripheral blood of endotoxin-shocked monkeys. Extensive leukopenia (WBC), and thrombopenia (platelets), increased alkaline phosphatase (ALP), as well as increased creatinine phosphokinase (CPK), were observed in blood from AcPepA-treated monkeys and in untreated monkeys following LPS injection. Esssentially no significant changes were observed in a monkey treated with AcPepA alone without LPS injection.*

C5a appears to exceed the capacity of CPR, since administration of lipopolysaccharide (LPS) at a lethal dose to rats exhausted CPR capacity before death (21). However, attempts to restrict the effect of C5a with C5a receptor (C5aR) antagonists would not be successful because C5aR is not only expressed on inflammatory leukocytes, but also on many other cell types (17). Furthermore, C5aR numbers increase in an acute inflammatory state (22).

On the other hand, antibodies to C5a have been demonstrated to be effective in treating experimental primate models of sepsis (16, 23), indicating that C5a inhibitors should be useful for treatment of patients suffering from hyperinflammation such as in sepsis and multiple organ failure (24). If an inhibitor of C5a has a therapeutic effect on sepsis, which impairs the status of cancer-bearing patients, the inhibitor could be beneficial for the cancer patients in order to improve their performance status.

AHB in C5aR, and between C5aR and C5a were analyzed by ANTIS program, and we found that amino acids 37 to 53 of C5a (RAARISLGPRCIKAFTE) comprise an antisense peptide to AHB peptides (9) of the C5aR, and this has been designated PL37 (25). This region of C5a is presumed to be a potential site for C5aR stimulation (26). Using the computer program MIMETIC (11), we generated 19 C-peps to PL37. One of the 7 inhibitory C-peps to PL37 which interfered with C5a function was termed PepA (ASGAPAPGPAGPLRPMF) (14). To improve stability, we modified PepA by acetylation of its *N*-terminal alanine generating acetylated PepA (AcPepA) which was more stable in animal experiments (27). In preliminary experiments with human lung tissues, AcPepA successfully suppressed the allergic response *in vitro* (28). Therefore, we performed experiments in *Cynomolgus* monkeys *in lieu* of using humans.

Table I. *Inhibitory capacity of complementary peptides (C-pep) to target molecules. Complementary peptides designed by MIMETIC program were synthesized and their inhibitory capacity on the function of target molecules determined. About 30% of these peptides interfered with the activity of target molecules.*

Target molecule	Activity of target	Number of C-pep	
		Tested	Effective (%)
HIV-RT*	Enzyme activity	10	3 (30%)
ProCPR **(TAFI)	Enzyme activity	10	3 (30%)
Thrombomodulin	Cofactor activity***	3	2 (67%)
C5a anaphylatoxin	Bioactivity	19	7 (37%)

*RT: Reverse transcriptase (12); **ProCPR: procarboxypeptidase R; ***cofactor activity for thrombin (13).

Materials and Methods

Peptides. PepA (ASGAPAPGPAGPLRPMF) whose *N*-terminal alanine is acetylated (AcPepA) was synthesized and purified (over 95% purity) by Biologica Co. Ltd. (Nagoya, Japan). The peptide was dissolved in saline at a concentration of 2 mg/ml and passed through a 0.22 μm Millipore filter prior to administration intravenously with an automated injection pump.

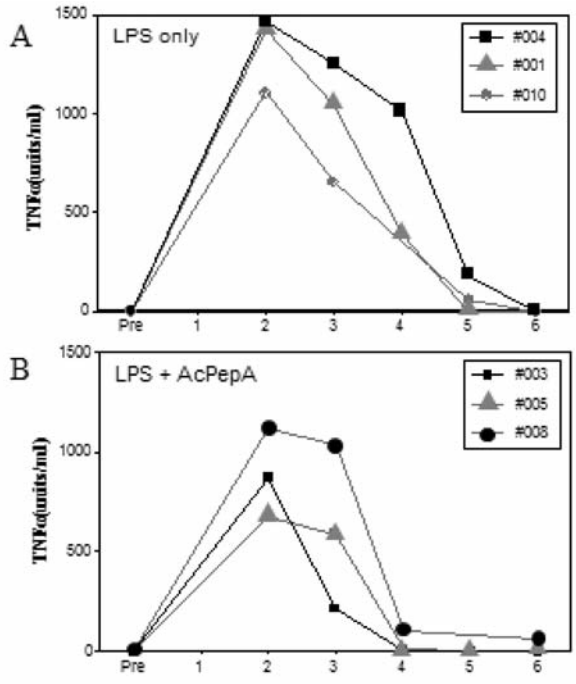

Figure 2. *TNFα levels in plasma of monkeys injected with 4 mg/kg LPS (A) and monkeys treated with AcPepA following the LPS injection (B). AcPepA t (2 mg/kg/h) was intravenously infused from 30 min to 6 h following LPS injection. AcPepA treatment suppressed the TNFα level to approximately 60% those of the untreated monkeys. TNFα in plasma disappeared within 4 h after LPS injection in AcPepA-treated monkeys, whereas this took 5 h in untreated monkeys.*

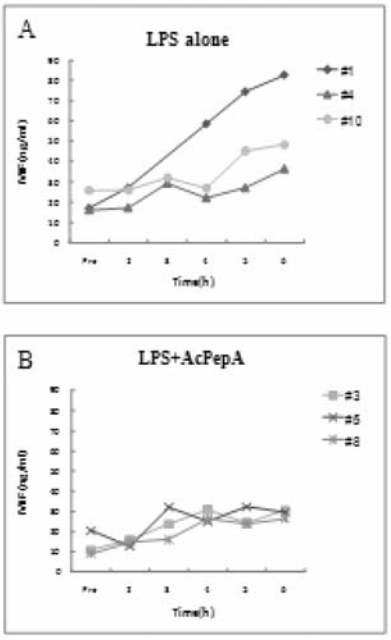

Figure 3. *Macrophage migration inhibitory factor (MIF) levels in plasma. Monkeys injected with LPS alone (#1, #4, #10; A), and monkeys treated with AcPepA following the LPS injection (#3, #5, #8; B) were tested for their MIF levels. MIF of AcPepA-treated monkeys remained at low levels (less than 30 ng/ml), whereas that of untreated monkeys increased to over 30 ng/ml following LPS injection.*

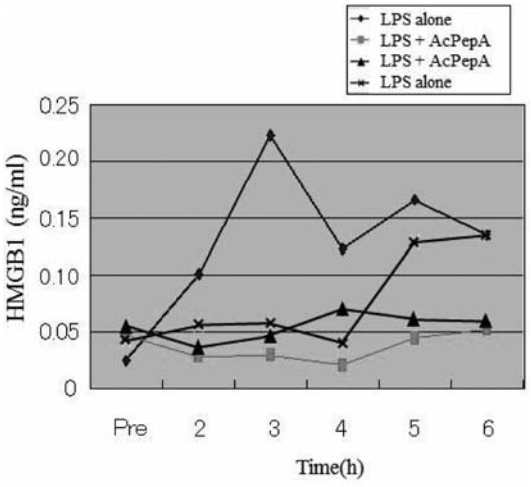

Figure 4. *Increase of HMGB1 in plasma of monkeys injected with LPS. Monkeys injected with LPS alone (#1, #10) showed increased HMGB1 levels in plasma, while AcPepA treatment following LPS injection (#5, #8) did not cause an increase (34).*

Monkeys. Cynomolgus monkeys were supplied from a breeding colony maintained at the Corporation for Production and Research of Laboratory Primates (CPRLP), Tsukuba, Japan. The Institutional Animal Ethical Committee of the Choju Medical Institute, Fukushimura Hospital, and the Institutional Animal Care Use Committees of the Tsukuba Primate Research Center, National Institute of Biomedical Innovation approved the study protocol. Animals weighed 4 to 5.5 kg, had hematocrits exceeding 36% and were free of infection, including tuberculosis. Animals were held for one month prior to LPS-lethal shock studies at CPRLP.

Titration of cytokine levels in plasma. TNFα in monkey plasma was determined using an ELISA kit purchased from Quantikine Immunoassay (Minneapolis, MN, USA). Macrophage inhibitory factor (MIF) was determined by use of an ELISA kit (29) prepared by Sapporo Immuno Diagnostic Laboratory (Sapporo, Japan). For high mobility group box 1 (HMGB1) determination, an ELISA kit from Shino-Test Co. (Sagamihara-shi, Kanagawa, Japan) was used.

Treatment of Cynomolgus monkeys. Fourteen monkeys were used for the experiment, and 13 were administered a lethal dose of LPS (4 mg/kg) sufficient to kill a monkey within 2 days, and 1 monkey was not administered LPS as an untreated control. Following sedation using ketamine hydrochloride (14 mg/kg, subcutaneously), monkeys were anesthetized with sodium pentobarbital administered through the capalic vein *via* a percutaneous catheter to maintain light surgical anesthesia. Oral intubation allowed animals to breathe spontaneously. Under anesthesia with sodium pentobarbital, 13 monkeys were intravenously administered 4 mg/kg LPS within 30 min. Thirty minutes after the LPS injection, 8 of the 14 animals were

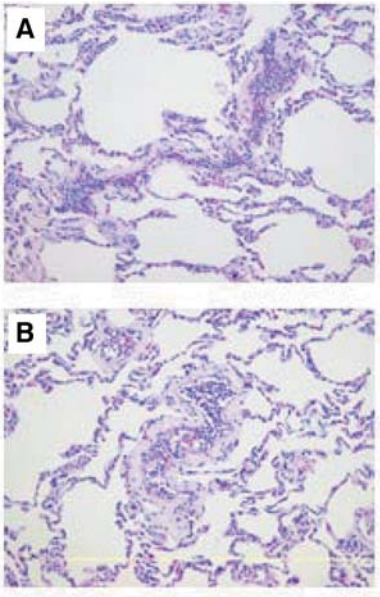

Figure 5. *Inflammation in lungs of monkeys 6 h after injection of a lethal dose of LPS observed in AcPepA-treated monkey (B) was to the same extent as that in untreated monkeys after LPS injection (A). (Magnification: ×200).*

administered 2 mg/kg of AcPepA in 2 min followed by 2 mg/kg/h of AcPepA for 3 h. The other 6 LPS-injected monkeys were injected with saline instead of AcPepA treatment as untreated controls. Six hours after the LPS administration, anesthesia was terminated and monkeys were returned to their cages to observe their status without any additional interference. However, half (3 animals) of the untreated controls and one of the AcPepA-treated monkeys were euthanized for autopsy for histopathological analysis of inflammation at 6 h after the LPS administration.

Results

Although all three monkeys administered saline alone as an untreated control died within two days (two in one day and one in two days), administration of 2 mg/kg of AcPepA in 2 min followed by 2 mg/kg/h of AcPepA for 3 h starting 30 min after the LPS injection rescued all of 7 monkeys who returned to a healthy condition in two days (Table II). Following LPS administration, significant leukopenia and thrombopenia were observed in peripheral blood obtained 6 h after the LPS injection both from monkeys treated with AcPepA and from control monkeys treated with saline instead of AcPepA (Figure 1). The increased TNFα level in plasma obtained during experiments in AcPepA-treated monkeys was lowered by only about 30% compared with that of untreated monkeys (Figure 2). The increase in the level of MIF (Figure 3) and HMGB1 (Figure 4) after LPS injection tended to be suppressed in the AcPepA-treated monkeys. Some of the monkeys were sacrificed under

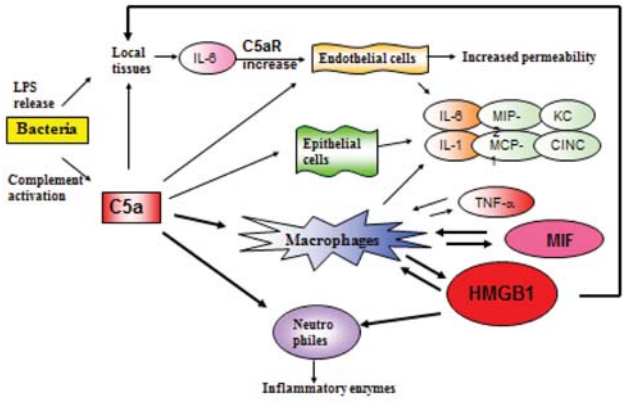

Figure 6. *Role of C5a anaphylatoxin in induction of an inflammatory cytokine 'storm'.*

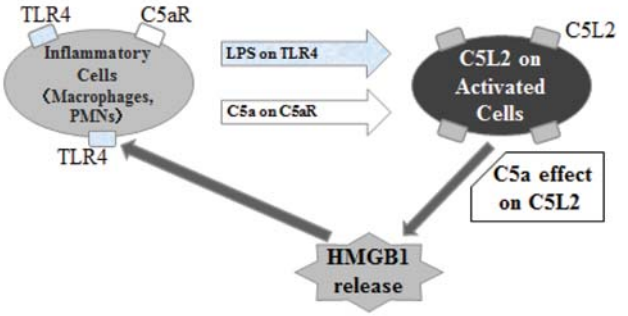

Figure 7. *Possible role for C5a in a positive feedback inflammatory circuit. Following bacterial infection, LPS stimulates TLR4, and C5a generated during complement activation stimulates C5aR, resulting in expression of C5L2 on leukocyte membranes. Stimulation of C5L2 by C5a on activated leukocytes 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 (34).*

anesthesia 6 h after LPS administration in order to perform autopsies. Pathological analysis of organ tissues showed serious inflammatory changes, including leukocyte infiltration, to the same extent in the lungs of both treated and untreated monkeys (Figure 5).

Discussion

The monkeys treated with AcPepA might have escaped induction of a feedback inflammatory circuit which was progressing gradually in LPS-treated monkeys at a late stage of the endotoxin shock syndrome as a vicious circle (Figure 6). This may be because HMGB1 has the capacity to stimulate toll-like receptor 4 (TLR4) and TLR2 as an endogenous stimulator (30, 31).

Table II. *Therapeutic effect of AcPepA on monkeys inoculated with a lethal dose of lipopolysaccharide (LPS) (4 mg/kg).*

	LPS alone	LPS and AcPepA	AcPepA alone
Decreased blood pressure	4/6	3/7	0/1
Increase in body temperature	5/6	3/7	0/1
Leukopenia	6/6	7/7	0/1
Increased CPK	6/6	7/7	0/1
Death	3/3	0/7	0/1
Euthanized at 6 h*	3	1	

CPK: Creatinine phosphokinase; *for histopathological analysis.

Consequently, inhibition of HMGB1 release presumably rescued animals suffering from septic vicious circle (32, 33). Therefore, suppression of HMGB1 in monkeys treated with AcPepA (Figure 4) could explain the extreme therapeutic effect of AcPepA on endotoxin shock in these animals (34). In other words, continuous generation of C5a by LPS or bacteria in monkeys receiving as well as possibly in patients with sepsis likely induce a cytokine 'storm' amplified by the release of HMGB1, resulting in a lethal effect on the host as a vicious circle of inflammation. The suppression of HMGB1 induction by inactivation of C5a could directly correlate with the survival observed following AcPepA treatment of monkeys injected with a lethal dose of LPS. Furthermore, AcPepA was shown to suppress pathophysiological events and prolonged survival time of piglets with sepsis induced by cecal ligation and perforation (CLP) (35, 36). Survival times were longer in the AcPepA-treated group than in the group treated with CLP alone (19.3 h±2.7 h *vs*. 9.9 h±0.7 h, $p<0.005$). In this case, AcPepA also delayed the HMGB-1 surge (36).

Therefore, suppression of C5 anaphylatoxin by AcPepA 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 (37-39), inhibition of C5a successfully interferes with the above release (Figure 7) (34).

Therefore, AcPepA would be beneficial for treatment of patients with sepsis and could be administered in large amounts at an acute stage, with little likelihood of an overdose, since the half-life of AcPepA in rats is 2.5 min. Administration of AcPepA to cancer patients at their terminal stage of their disease could improve their performance status.

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References

1 Blalock J E and Smith EM: Hydropathic anti-complementarity of amino acids based on the genetic code. Biochem Biophys Res Comm *121*: 203-207, 1984.
2 Blalock JE: Complementarity of peptides specified by 'sense' and 'antisense' strands of DNA. Trends Biotech *6*: 140-144, 1991.
3 Trospha A, Kizer JS and Chaiken IM: Making sense from antisense: A review of experimental data and developing ideas on sense-antisense peptide recognition. J Molec Recognition *5*: 43-53, 1992.
4 Eberle AN and Huber MJ: Antisense peptides: Tools for receptor isolation? Lack of antisense MSH and ATH to interact with their sense peptides and induce receptor specific antibodies. Receptor Res *11*: 13-43, 1991.
5 Rassmundsen UB and Hesh RD: Antisense peptides: the parathyroid hormone as an experimental example and a critical theoretical view. Biochem Biophys Res Comm *149*: 930-938, 1987.
6 Goldstein A and Brutlag DL: Is there a relationship between DNA sequences encoding peptide ligands and their receptors? Proc National Acad Sci USA *86*: 42-45, 1989.
7 Guillemette G, Boulay G, Gagnon S, Bosse R and Escher E: The peptide encoded by angiotensin II complementary RNA does not interfere with angiotensin II action. Biochem J *261*: 309, 1989.
8 de Gasparo M, Whitebread S, Einsle K and Heusse C: Are the antibodies to a peptide complementary to angiotensin II useful to isolate angiotensin II receptor? Biochem J *261*: 310-311, 1989.
9 Baranyi L, Campbell W, Ohshima. K, Fujimoto S, Boros M and Okada H: The antisense homology box: a new motif within proteins that encodes biologically active peptides. Nature Med. *1*: 894-901, 1995.
10 Baranyi L, Campbell W, Oshima K, Fujimoto S, Boros M, Kaszaki J and Okada H: Antisense homology box-derived peptides represent a new class of endothelin receptor inhibitors. Peptides *19*: 211-223, 1998.
11 Campbell W, Kleiman L, Baranyi L, Khorchid Z, Li A, Fujita E, Okada N and Okada H: A novel genetic algorithm for designing mimetic peptides that interfere with the function of a target molecule. Microbiol Immunol *46*: 211-215, 2002.
12 Hosokawa M, Imai M, Okada H and Okada N: Inhibition of HIV-1 infection in cells expressing an artificial complementary peptide. Biochem Biophys Res Comm *324*: 236-240, 2004.
13 Shimomura Y, Kawamura T, Komura H, Campbell W, Okada N and Okada H: Modulation of procarboxypeptidase R (proCPR) activation by complementary peptides to thrombomodulin. Microbiol Immunol *47*: 241-245, 2003.
14 Fujita E, Farkas I, Campbell W, Baranyi L, Okada H and Okada N: Inactivation of C5a anaphylatoxin by a peptide that is complementary to a region of C5a. J. Immunol *172*: 6382-6387, 2004.
15 Ember JA and Hugli TE: Complement factors and their receptors. Immunopharmacology *38*: 3-15, 1997.
16 Czermak BJ, Sarma V, Pierson CL, Warner RL, Huber-Lang M, Bless N.M, Schmal H, Friedl HP and Ward PA: Protective effects of C5a blockade in sepsis. Nature Med *5*: 788-792, 1999.
17 Guo RF and Ward PA: Role of C5a in inflammatory responses. Annu Rev Immunol *23*: 821-852, 2005.

18 Michael K: Targeting complement in therapy. Immunol Rev *180*: 177-189, 2001.
19 Campbell W, Lazoura E, Okada N and Okada H: Inactivation of C3a and C5a octapeptides by carboxypeptidase R and carboxypeptidase N. Microbiol Immunol *46*: 131-134, 2002.
20 Campbell W, Okada N and Okada H: Carboxypeptidase R (CPR) is an inactivator of complement derived inflammatory peptides and an inhibitor of fibrinolysis. Immunol Rev *180*: 162-167, 2001.
21 Kato K, Shinagawa N, Hayakawa T., Takeyama H, Campbell W and Okada H: Changes in arginine carboxypeptidase (CPR) activity in stressed rats. Pathophysiology *1*: 131-136, 1994.
22 Riedemann NC, Guo RF, Neff TA, Laudes IJ, Keller KA, Sarma VJ, Markiweski MM, Mastellos D, Strey CW, Pierson CL, Lambris JD, Zetoune FS and Ward PA: Increased C5a receptor expression in sepsis. J Clin Invest *110*: 101-108, 2002.
23 Stevens JH, O'Hanley P, Shapiro JM, Mihm FG, Satoh PS, Collins JA and Raffin TA: Effects of anti-C5a antibodies on the adult respiratory distress syndrome in septic primates. J Clin Invest *77*: 1812-1816, 1986.
24 Riedemann NC, Guo RF and Ward PA: Novel strategies for the treatment of sepsis. Nature Med *9*: 517-524, 2003.
25 Baranyi L, Campbell W and Okada H: Antisense homology boxes in C5a receptor and C5a anaphylatoxin. J Immunol *157*: 4591-4601, 1996.
26 Farkas I, Baranyi L, Liposits ZS, Yamamoto T and Okada H: Complement C5a anaphylatoxin fragment causes apoptosis in TGW neuroblastoma cells. Neuroscience *86*: 903-911, 1998.
27 Okada N, Asai S, Hotta A, Miura N, Ohno N, Farkas I, Hau L and Okada H: Increased inhibitory capacity of an anti-C5a complementary peptide following acetylation of *N*-terminal alanine. Microbiol Immunol *51*: 439-443, 2007.
28 Abe M, Hama H, Shirakusa T, Iwasaki A, Ono N, Kimura N, Hugli TE, Okada N, Katsuragi T and Okada H: Contribution of anaphylatoxins to allergic inflammation in human lungs. Microbiol. Immunol *49*: 981-986, 2005.
29 MizueY, Nishihira J, Miyazaki T, Fujiwara S, Chida M, NakamuraK, Kikuchi K and Mikai M: Quantitation of macrophage migration inhibitory factor (MIF) using the one-step sandwich enzyme immunosorbent assay: elevated serum MIF concentrations in patients with autoimmune diseases and identification of MIF in erythrocytes. Int J Mol Med *5*: 397-403, 2000.
30 Park JS, Gamboni-Robertson F, He Q, Svetkauskaite D, Kim JY, Strassheim D, Sohn JW, Yamada S, Maruyama I, Banerjee A, Ishizaka A and Abraham E: High mobility group box 1 protein interacts with multiple Toll-like receptors. Am J Physiol Cell Physiol *290*: C917-924, 2006.
31 Yu M, Wang H, Ding A, Golenbock DT, Latz E, Czura CJ, Fenton MJ, Tracey KJ and Yang H: HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. Shock 26: 174-179, 2006.
32 Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, Frazier A, Yang H, Ivanova S, Borovikova L, Manogue KR, Faist E, Abraham E, Andersson J, Andersson U, Molina PE, Abumrad NN, Sama A and Tracey KJ: HMGB-1 as a late mediator of endotoxin lethality in mice. Science *285*: 248-251, 1999.
33 Yang H, Ochani M, Li J, Qiang X, Tanovic M, Harris H, Susaria SM, Ulloa MP, Wang H, DiRimo R, Czura CJ, Wang H, Roth J, Warren HS, Fink MP, Fenton MJ, Andersson U and Tracey KJ. Reversing established sepsis with antagonists of endogeneous high-mobility group box 1. Proc Natl Acad Sci *101*: 296-301, 2004.
34 Okada N, Imai M, Okada A, Ono F and Okada H: HMGB1 release by C5a anaphylatoxin is an effective target for sepsis treatment. Nature Precedings, (hdl:10101/npre.2011.5727.1: Posted 23 Feb 2011) 2011.
35 Hussein MH, Kato S, Goto T, Daoud GA, Kato I, Suzuki S, Togari H, Imai M, Okada N and Okada H: An acetylated anti-C5a complementary peptide reduced cytokines and free radicals and prolongs survival time in a neonatal sepsis model Mol Immunol *46*: 2825, 2009.
36 Hussein MH, Daoud G Al-H, Goto T, Kato S, Nobata M, Kakita H, Mizuno H, Ito T, Kato I, Suzuki S, Okada N, Hashimoto T, Okada H and Togari H: An acetylated anti-C5a complementary peptide reduced cytokines and free radicals and prolonged survival time in a neonatal sepsis model. Critical Care Medicine, Submitted, 2011.
37 Chen NJ, Mirtsos C, Suh D, Lu YC, Lin WJ, McKerlie C, Lee T, Baribault H, Tian H and Yeh WC: C5L2 is critical for the biological activities of the anaphylatoxins C5a and C3a. Nature *446*: 203-207, 2007.
38 Klune JR, Dhupar R, Cardinal J, Billiar TR and Tsung A: HMGB1: Endogeneous danger signaling. Mol Med *14*: 476-484, 2008.
39 Rittirsch D, Flierl MA, Nadeau BA, Day DE, Huber-Lang M, Mackay CR, Zetoune FS, Gerard NP, Cianflone K, Koehl J, Gerard C, Sarma JV and Ward PA: Functional roles for C5a receptors in sepsis. Nature Med *14*: 551-557, 2008.

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