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Foot notes

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

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[3]Abbreviations used in this paper: PepA, name of an inhibitory peptide of C5a; AcPepA, acetylated PepA; C5aR, C5a receptor; CRP, carboxypeptidase R; HMGB1, high mobility group box 1 ; PL37, amino acid 37 to 53 of C5a; C5L2, newly found C5a receptor.

Table 1. Therapeutic effect of AcPepA on monkeys inoculated with a lethal dose of LPS

LPS-injected monkeys treated with	Number of animals per total: leucopenia (<2000/mm^3)	CPK increase (>50 u/ml)	Survivor/total (%)
Saline (untreated control)	3/3	3/3	0/3 (0%)
AcPepA (2mg/kg/hr)	7/7	7/7	7/7 (100%)

Following sedation using ketamine hydrochloride (14mg/kg, subcutaneously), monkeys were anesthetized with sodium pentobarbital administered through the capalic vein via a percutaneous catheter to maintain light level surgical anesthesia. Oral intubation allowed animals to breathe spontaneously. Under anesthesia, monkeys were intravenously administered 4 mg/kg LPS within 30 min. Six hours after LPS administration, anesthesia was terminated and monkeys were returned to their cages without any additional interference.

Although all 3 monkeys administered saline alone as an untreated control died within 2 days (two in one day and one in two days), administration of 2mg/kg of

AcPepA in 2 min followed by 2 mg/kg/hr of AcPepA for 3 hrs starting 30 min after the LPS injection rescued all of 7 monkeys who returned to a healthy condition in 2 days. Following LPS administration, significant leukopenia and thrombopenia were observed 6 hrs after the LPS injection even in monkeys treated with AcPepA (Figure 1). However, the TNFα level in plasma obtained during experiments in AcPepA treated monkeys decreased by about 40% compared with that of untreated monkeys. Although the serum level of HMGB1 increased after LPS injection, the HMGB1 level did not increase in the LPS shock monkeys treated with AcPepA (Figure 2). Some monkeys were sacrificed under anesthesia 6 h after LPS injection for autopsies. Serious inflammatory changes including leukocyte infiltration were observed to the same extent in the lungs of both AcPepA treated and untreated monkeys.

Figure 1. **Clinical features in peripheral blood of endotoxin-shock 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 (red) following LPS injection, as in the case of untreated LPS-injected monkeys (blue). Essentially no significant changes were observed in a monkey (yellow) treated with AcPepA alone.

Figure 2. Increase in HMGB1 in plasma of LPS- injected monkeys.

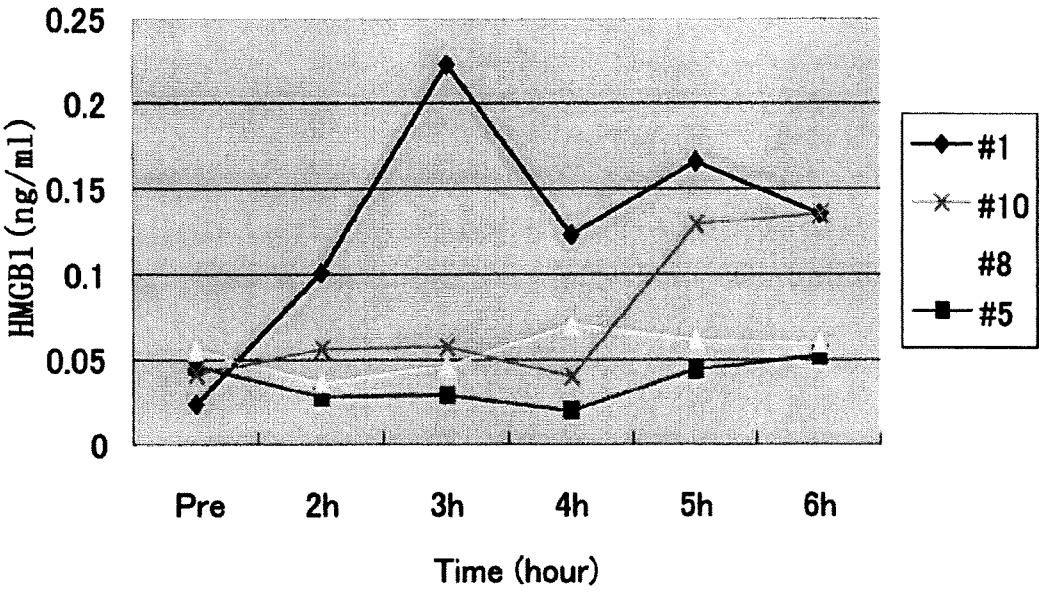
Monkeys injected with LPS alone (#1, #10) showed increased HMGB1 levels in plasma, while AcPepA-treated monkeys did not show increase following LPS injection (#5, #8).

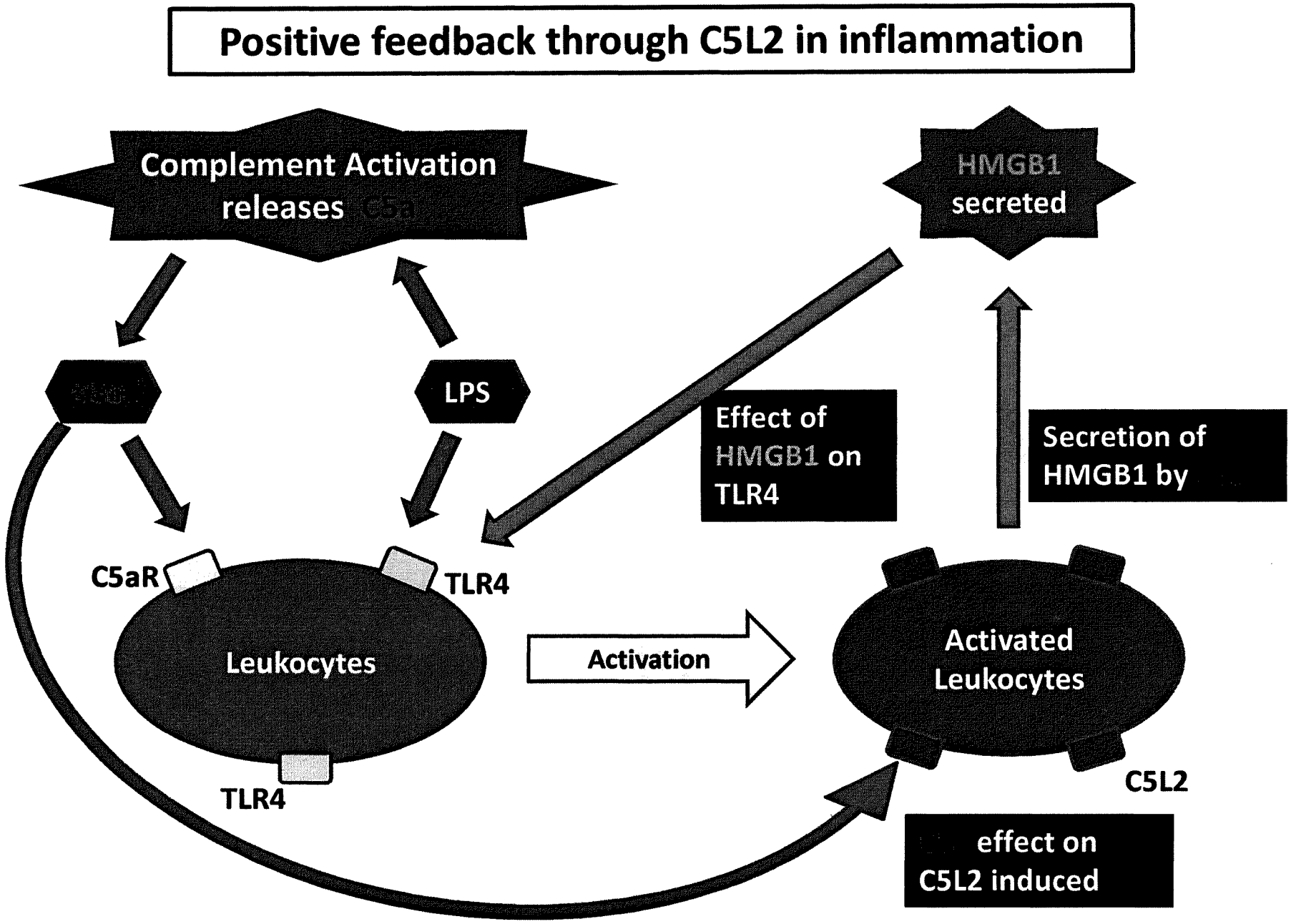
Figuere 3. Possible role for C5a in a positive feedback inflammatory circuit.

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.

Platelets
x10³/µl
Time (hr.)
CPK
IU/L
Time (hr.)
WBC
x10³/µl
Time (hr.)
ALP
IU/L
Time (hr.)

(Fig. 2 Okada et al.)




Positive feedback through C5L2 in inflammation
Complement Activation releases C5a
HMGB1 secreted
LPS
Effect of HMGB1 on TLR4
Secretion of HMGB1 by
C5aR
TLR4
Leukocytes
TLR4
Activation
Activated Leukocytes
C5L2
effect on C5L2 induced