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Immunomodulatory Actions of Lysophosphatidylcholine

Chang-Won HONG and Dong-Keun SONG*

Department of Pharmacology, Institute of Natural Medicine, Infectious Disease Medical Research Center, College of Medicine, Hallym University, Chunchon 200-702, South Korea

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Abstract – Lysophosphatidylcholine (LPC) is an endogenous phospholipid. LPC has various stimulating or modulating activities on immune cells, including lymphocytes, monocytes/macrophages and neutrophils. Studies generally revealed pro-inflammatory activities of LPC, but accumulating evidence indicates that LPC has also anti-inflammatory actions. Here we summarize immunomodulatory actions of LPC.

Keywords: Lysophosphatidylcholine, Lymphocyte, Monocyte, Macrophage, Neutrophil

INTRODUCTION

Lysophosphatidylcholine (LPC) is an endogenous phospholipid. The concentration of LPC in plasma is very high, around 200-300 μM, with most LPC bound to albumin and lipoprotein (Kishimoto *et al.,* 2002). Because LPC is a major component of oxidized low-density lipoprotein (oxLDL), an important pathogenic factor for atherosclerosis, studies have been largely focused on LPC actions on cells involved in atherosclerosis.

Atherosclerosis is a long-standing mild inflammatory process. In addition to its effects on endothelial and smooth muscle cells, LPC has various stimulating or modulating activities on immune cells, including lymphocytes, monocytes/macrophages and neutrophils. These studies generally revealed pro-inflammatory activities of LPC, but accumulating evidence indicates that LPC has also anti-inflammatory actions, making its profile more complex than initially thought. Here we summarize immunomodulatory actions of LPC (Table I).

LYMPHOCYTES

LPC (C14:0, C16:0, C18:0, 10-50 μM) greatly increases the activation of human resting T lymphocytes, as measured by the expression of the α subunit of IL-2 receptor and thymidine uptake when co-stimulated with diacylglycerol or phorbol 12-myristate 13-acetate (PMA) plus ionomycin, a calcium ionophore, whereas LPC alone has no effect (Asaoka *et al.,* 1991; Asaoka *et al.,* 1992). LPC (C16:0; 37.5, 50 μM) induces gene expression and secretion of heparin-binding epidermal growth factor-like growth factor (HB-EGF), a smooth muscle growth factor (Nishi *et al.*, 1997), and enhances cytokine-induced interferon (IFN)-γ expression (Nishi *et al.,* 1998) in human T (CD4+ and CD8+) lymphocytes. LPC (C16:0, 1, 30 μM) up-regulates CD40 ligand expression in anti-CD3 antibody and CD80-stimulated human CD4+ T cells (Sakata-Kaneko *et al.,* 1998).

LPC (C16:0, 1-50 μM) is chemoattractant for human T lymphocytes (McMurray *et al.*, 1993; Ryborg *et al.,* 1994). A study using a T-cell line (DO 11.10 T cell hybridoma) showed that LPC-induced chemotaxis is via G2A (Radu *et al.*, 2004).

In addition to its chemotactic activity toward T cells, LPC up-regulates the expression of CXCR4, a chemokine receptor, in human CD4+ T cells (Han *et al.,* 2004). Thus, LPC induces T cell chemotaxis to SDF-1, a ligand for CXCR4, and enhances SDF-1-induced IL-2 and IFN-γ release from CD4+ cells activated by anti-CD3 antibody (Han *et al.,* 2004).

LPC also affects B lymphocyte function; in peripheral blood mononuclear cells (PBMC), LPC (16:0, 1-25 μM) increases antibody formation, most notably IgA (Huang *et al.*, 1999).

LPC (a mixture of 16:0, 18:0 and 18:1, 5-50 μM) increases apoptosis and reactive oxygen species (ROS) generation in phytohemagglutinin (PHA)-activated but not in control human peripheral blood lymphocytes (Zurgil *et al.,* 2007).

LPC (20-45 μg/ml) induces $p56^{lck}$ and ZAP^{70} phosphorylation, and increases $[Ca^{2+}]_i$ in Jurkat cells, a cell line for

*Corresponding author
Tel: +82-33-248-3290, Fax: +82-33-248-3293
E-mail: dksong@hallym.ac.kr

Table I. Some important actions of lysohosphatidylcholine on lymphocyte, monocyte/macrophage, and neutrophil

Lymphocyte
Chemoattractant (McMurray *et al.,* 1993; Ryborg *et al.,* 1994; Radu *et al.,* 2004)
IL-2Rα↑/thymidine uptake↑ (Asaoka *et al.,* 1991; Asaoka *et al.,* 1992)
HB-EGF↑ (Nishi *et al.,* 1997), IFN-γ↑ (Nishi *et al.,* 1998)
CD40 ligand↑ (Sakata-Kaneko *et al.,* 1998), CXCR4↑, IL-2↑ (Han *et al.,* 2004)
Antibody formation↑, most notably IgA (Huang *et al.,* 1999)
Apoptosis↑/ROS generation↑ (Zurgil *et al.,* 2007)
$p56^{lck}$ and ZAP^{70} phosphorylation↑, $[Ca2+]_i$↑ in Jurkat cells (Legradi *et al.,* 2004)
Monocyte/macrophage
Chemoattractant (Quinn *et al.,* 1988)
Activates p38 and p42/44 MAPKs in THP-1 cells (Jing *et al.,* 2000)
Mitogenic effects of acetylated LDL↑ (Sakai *et al.,* 1994)
Mature dendritic cell generation↑/CD86↑ (Coutant *et al.,* 2002)
Adhesion↑/activation of CD11b (Weber *et al.,* 1995)
IFN-γ↑ (Nakano *et al.,* 1994), IL-1 β↑ (Liu-Wu *et al.,* 1998), TNF-α↑ (Huang *et al.,* 1999), MIP2↑ in RAW264.7 cells (Olofsson *et al.,* 2008), arachidonic acid ↑ in THP-1 and Mono Mac6 cells (Oestvang *et al.,* 2003)
Phospholipase D (PLD) activity ↑ (Gómez-Muñoz *et al.,* 1999)
Extracellular acidification ↑ in RAW264.7 cells (De Vries *et al.,* 1998)
EC-SOD ↑ in U937 cells (Yamamoto *et al.,* 2002)
Ca^{2+} influx ↑ in U937 human monocytes (Yun *et al.,* 2004)
De-ramification of murine microglia↑/nonselective cation current↑, calcium-activated potassium current↑/ hyperpolarization↑ (Schilling *et al.,* 2004)
Neutrophil
Synergistically enhances PMA-induced superoxide anion generation (Englberger *et al.,* 1987)
Generation from stored blood (Silliman *et al.*, 1994; Silliman *et al.,* 1996)
Superoxide anion generation is inhibited with PI3K inhibitors (Nishioka *et al.,* 1998)
H_2O_2 generation/bactericidal activity in a G2A-dependent manner (Yan *et al.*, 2004)
Priming of NADPH oxidase is dependent on $[Ca^{2+}]_i$ increase (Silliman *et al.,* 2003)
Apoptosis↓ (Biffl *et al.,* 2003)
$[Ca^{2+}]_i$ ↑ via G2A/mobilization of G2A along with secretory vesicles (Frasch *et al.,* 2007)
$[Ca^{2+}]_i$ ↑ via PLC in HL60 cells (Okajima *et al.,* 1998)

human CD4+ T lymphocytes (Legradi *et al.,* 2004). The LPC-induced $[Ca^{2+}]_i$ level is blocked by pertussis toxin (PTX), a Gi blocker, genistein, a tyrosine kinase inhibitor and staurosporine, a PKC inhibitor. In S49 lymphoma cells, LPC (C16:0, 2-25 μM)-induced Ca^{2+} increase by formation of transient non-protein calcium pores was reported (Wilson-Ashworth *et al.,* 2004).

MONOCYTES/MACROPHAGES

LPC (16:0, 25 μM) induces chemotaxis of human monocytes (Quinn *et al.,* 1988). As for T lymphocytes, LPC-mediated chemotaxis of macrophages is mediated via G2A, shown in mouse peritoneal macrophages and J774A.1 macrophages (Yang *et al.,* 2005). Interestingly, unlike other chemoattractants which are dependent on Gi proteins, G2A-mediated macrophage chemotaxis uses Gq/11 and G12/13 (Yang *et al.*, 2005). However, in $G2A^{-/-}$ mice, infiltration of macrophages and T lymphocytes was not reduced in atherosclerotic lesion (Parks *et al.,* 2005).

LPC released by apoptotic cells is a phagocyte attraction signal; LPC (10-20 μM) was chemotactic to THP-1 and mono Mac 1 cells (Lauber *et al.,* 2003). In studies using U937 (G2A transfected), THP-1, and mouse J774A.1

macrophages, chemotaxis of these cells to LPC is mediated by G2A (Peter *et al.,* 2008).

In THP-1 cells, LPC (25 μg/ml) activates p38 and p42/44 mitogen-activated protein kinases (MAPKs) but only p38 activation is involved in stimulated chemotaxis (Jing *et al.,* 2000). LPC-induced p38 phosphorylation is blocked by PTX, and inhibited by a phospholipase C inhibitor (U73122) and phosphatidylinositol 3-kinase (PI3K) inhibitors (wortmannin and LY294002).

LPC (C16:0, C18:0, C18:1, 50 μM) markedly enhances mitogenic effects of acetylated LDL on murine resident macrophages, whereas LPC alone has no effect (Sakai *et al.,* 1994).

LPC (40 μM) promotes mature dendritic cell generation from differentiating monocytes with up-regulation of CD86. LPC-induced CD86 up-regulation is inhibited by platelet activating factor receptor (PAFR) antagonist, BN52021, and PTX (Coutant *et al.,* 2002). LPC-treated mature dendritic cells induce a Th1-biased response, partly via the peroxisome proliferator-activated receptors (PPAR) signaling (Coutant *et al.,* 2004).

LPC increases adhesion of human peripheral blood monocytes. This effect is mediated by activation of CD11b via protein kinase C (PKC) signaling (Weber *et al.,* 1995).

As the case for T lymphocytes (Nishi *et al.,* 1997), LPC (C18:0, 25 and 37.5 μg/ml) increases the expression of HB-EGF in human monocytes (Nakano *et al.,* 1994). LPC (C16:0, 25, 30 μM) induces the production of IL-1β in human monocytes (Liu-Wu *et al.,* 1998) and in mouse peritoneal macrophages (Yan *et al.,* 2004). LPC (C16:0, 5 μM) induces the production of IFN-γ and tumor necrosis factor (TNF)-α from human PBMC (Huang *et al.,* 1999). This response is inhibited by WEB 2170, a PAFR antagonist (Huang *et al.,* 1999). LPC induces increased release of macrophage-inflammatory protein-2 (MIP2) and mRNA expression from RAW264.7 cells (Olofsson *et al.*, 2008).

LPC itself is a product of phospholipase A2 (PLA_2). However, intriguingly, LPC (C16:0, 40 μM) induces arachidonic acid release from THP-1 and Mono Mac6 cells via secretary PLA_2 and cytosolic PLA_2 signal in a PTX-dependent manner (Oestvang *et al.,* 2003), suggesting that LPC somehow stimulates PLA_2, forming a positive feedback for generation of LPC. LPC (1 μg/ml) increases phospholipase D (PLD) activity in mouse peritoneal macrophages. This effect was inhibited by down-regulation of PKC activity (with prolonged incubation with PMA), or inhibitors of tyrosine kinase (genistein) and PAFR (WEB-2086) (Gómez-Muñoz *et al.,* 1999). Because PLD activity produces phosphatidic acid which can activate PLA_2 (Bauldry *et al.,* 1997), PLD could be involved in LPC-induced release of arachidonic acid from THP-1 and Mono Mac6 cells (Oestvang *et al.,* 2003).

LPC (10-100 μM) increases extracellular acidification in RAW264.7 cells (De Vries *et al.*, 1998). LPC (10-30 μg/ml) increases extracellular-superoxide dismutase (EC-SOD) expression and release from human monocytic U937 cells (Yamamoto *et al.,* 2002). In U937 cells, LPC (C14:0, 20 μM) did not induce significant Ca^{2+} influx (Lee *et al.,* 2004), but LPC (C16:0, C18:0, 10 μM) induced Ca^{2+} influx in U937 human monocytes in a PTX-insensitive manner (Yun *et al.,* 2004). In the subsequent study, LPC (C16:0, 10 μM)-induced Ca^{2+} influx was suggested to be via L-type calcium channel, as it was inhibited by verapamil (100 μM) and nifedifine (100 μM) (Lee *et al.,* 2006).

LPC (C16:0, 15 μM) induces de-ramification of murine microglia, and nonselective cation current, and calcium-activated potassium current, with the subsequent hyperpolarization (Schilling *et al.,* 2004)

NEUTROPHILS

LPC (1 μM) alone slightly increases superoxide anion generation, and when co-treated with PMA, synergistically enhances PMA-induced superoxide anion generation (Englberger *et al.,* 1987). Subsequent study showed that LPCs having fatty acids with more than 10 carbons enhance superoxide responses of stimulated human neutrophils (Ginsburg *et al.,* 1989; Wyman *et al.,* 2002). Lipids generated from stored blood were found to prime neutrophils, and identified to be LPCs (Silliman *et al.,* 1994; Silliman *et al.,* 1996).

LPC (C16:0, 5-15 μM)-induced superoxide anion generation is inhibited with PI3K inhibitors (wortmannin and LY294002), but not by PKC inhibitors (GF109203X and calphostin C) (Nishioka *et al.,* 1998). LPC (18:0, 30 μM) induces H_2O_2 generation and enhances bactericidal activity in murine neutrophils in a G2A-dependent manner (Yan *et al.,* 2004).

LPC-induced changes in human neutrophils were extensively studied by Silliman *et al.* (2003). In addition to priming formyl-Met-Leu-Phe (fMLP)-induced activation of the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, LPC (0.45-14.5 μM) enhances adhesion of neutrophils to fibrinogen-coated microtiter plates and surface expression of the CD11b and fMLP receptor (Silliman *et al.,* 2003). LPC (0.45 and 4.5 μM) increases $[Ca^{2+}]_i$ via PTX-sensitive manner, and migration of neutrophils in a Boyden chamber (Silliman *et al.,* 2003). LPC (4.5 μM) modestly increases release of elastase, myeloperoxidase, and lactoferrin (Silliman *et al.,* 2003).

All the above effects of LPC were blocked by chelating cytosolic calcium (Silliman *et al.,* 2003). LPC (4.5 μM) delays neutrophil apoptosis (Biffl *et al.,* 2003). LPC (C18:1, 10 μM) increases $[Ca^{2+}]_i$ in neutrophils via G2A, and induces mobilization of G2A along with secretory vesicles (Frasch *et al.,* 2007).

In HL60 leukemic cells, LPC (C16:0, 30 μM) increases $[Ca^{2+}]_i$ by stimulation of PLC in a PTX-sensitive manner, whereas LPC (C18:0, 30 μM), which *per se* very slightly increases $[Ca^{2+}]_i$, inhibits the effect of LPC (C16:0, 30 μM) (Okajima *et al.,* 1998). In the same cell line, a slight discrepancy was reported in that LPC (C18:0, 10 μM) and LPC (C16:0, 10 μM) show equipotent activity regarding $[Ca^{2+}]_i$ increase, although the onset of $[Ca^{2+}]_i$ increase induced by LPC (C18:0, 10 μM) is slower than LPC (C16:0, 10 μM)-induced one (Lee *et al.,* 2004).

For eosinohils, enhanced eosinophil adhesion by LPC via a non-store-operated Ca^{2+} influx leading to CD11b/CD18 activation was reported (Zhu *et al.,* 2007).

LPC concentrations

Most studies on LPC were conducted with LPC concentrations of around 1-50 μM. Recently, nanomolar LPC was shown to induce THP-1 monocyte chemotaxis (Olofsson *et al.,* 2008).

Because LPC avidly binds to albumin and lipoproteins, LPC activities are varied in different medium conditions, including the presence and the amount of albumin and serum in the medium. Therefore, not knowing the concentration of free LPC, comparison between data from different papers based only on the LPC concentrations used may be difficult to interpret.

Priming

One of characteristics of LPC actions is that LPC *per se* is a weak stimulator or even inactive, however, when LPC is combined with other stimulators, synergistic interactions often occurs (Englberger *et al.,* 1987; Ginsburg *et al.,* 1989; Asaoka *et al.,* 1991; Asaoka *et al.,* 1992; Sakai *et al.,* 1994; Wyman *et al*., 2002). The most prominent example is seen in the priming of respiratory burst of neutrophils. This LPC-induced priming was clearly shown to be due to the LPC-induced $[Ca^{2+}]_i$ increase (Silliman *et al*., 2003). LPC also increases $[Ca^{2+}]_i$ in other types of immune cells (Legradi *et al.,* 2004; Wilson-Ashworth *et al.,* 2004). This LPC-induced $[Ca^{2+}]_i$ increase most probably underlie the synergistic interactions between LPC and other stimulators observed also in other cells, including lymphocytes, and monocytes/macrophages (Asaoka *et al*., 1991; Asaoka *et al*., 1992; Sakai *et al.,* 1994; Schilling *et al.,* 2004; Yun *et al.,* 2004).

Anti-inflammatory actions of LPC

Although a number of papers demonstrated the pro-inflammatory actions of LPC, accumulating evidence shows that LPC has also anti-inflammatory profiles.

Systemic administration of LPC (18:0; 10 mg/kg s.c., four times every 12 h) to mice protected LPS-induced lethality, and inhibited LPS-induced increased in plasma levels of TNF-α and IL-1β (Yan *et al.,* 2004). *In vitro*, LPC inhibited basal and LPS-induced TNF-α release from neutrophils (Yan *et al.,* 2004). LPC administration inhibited LPS- or CLP-induced increase in plasma HMGB1 levels, and LPC inhibited high-mobility group box 1 (HMGB1) release from macrophages or monocytes (Chen *et al.,* 2005). Furthermore, LPC administration inhibited LPS- or peptidoglycan/lipoteichoic acid-induced multiple organ damage in rats (Murch *et al.,* 2006).

Several studies have shown that LPC can inhibit some lipopolysaccharide (LPS) actions. LPC (1-10 μM) inhibits LPS-induced tissue factor (TF) activity and mRNA expression from human monocytes (Engelmann *et al.,* 1999). LPC increases cAMP levels in monocytes (Engelmann *et al*., 1999) and THP-1 cells (Yuan *et al.,* 1996).

LPC (C16:0) inhibits PMA- or fMLP-stimulated ROS in neutrophils (Müller *et al*., 2002). Further, fMLP-induced ROS and other fMLP-induced signaling in neutrophils, as well as lung injury induced by fMLP-stimulated neutrophils were inhibited by LPC (0.1, 1 μM) treatment through elevation of cAMP (Lin *et al.,* 2005). In HL60 leukemic cells, an inhibitory effect of LPC (C18:0) on stimulated PLC-$[Ca^{2+}]_i$ signal was reported (Okajima *et al.,* 1998).

Because cAMP-PKA system negatively regulates inflammatory processes in immune cells, LPC-induced increase in cAMP (most probably via GPCR-Gs protein signaling) may underlie, at lest in part, some of the negative regulatory actions cited above.

In a similar vein, LPC (10 μg/ml) increases expression and release of EC-SOD, a protecting factor against atherogenesis, from human monocytic U937 cells (Yamamoto *et al*., 2002), possibly negating, to some extent, the pro-atherogenic effects of LPC.

LPC/PC ratio

Lysophosphatidylcholine acyltransferase (LPCAT) acylates LPC to phosphatidylcholine (PC), making LPC/PC ratio in the plasma to decrease. LPCAT inhibition (thus increasing LPC/PC ratio) inhibits LPS signaling (Schmid *et al.,* 2003). LPCAT inhibition (thus increasing LPC/PC ratio) prevents translocation of Toll-like receptor (TLR)-4,

the receptor for LPS, to lipid raft domain (Jackson *et al.*, 2008), supplying additional plausible evidence to explain the previous data of LPC inhibition of LPS signal.

In line with this contention, plasma levels of LPC are decreased in septic patients, and the decrease is greater in non-survivals (Drobnik *et al.,* 2003), suggesting the possibility that depletion of LPC may aggravate inflammatory process. Further supporting this notion, plasma levels of LPC are correlated inversely with inflammatory parameters, such as plasma C-reactive protein and whole blood hydrogen peroxide levels in cancer patients (Taylor *et al.,* 2007).

Receptors

G2A is expressed in lymphocytes (Weng *et al.*, 1998), macrophages (Rikitake *et al.,* 2002), and neutrophils (Yan *et al.,* 2004), but not U937 human monocytes (Yun *et al.,* 2004). A number of LPC actions, but not all, are blocked by either anti-G2A antibody or silencing of G2A (Han *et al.,* 2004; Radu *et al.,* 2004; Frasch *et al.,* 2007; Peter *et al.,* 2008). Therefore, G2A is regarded as an effector of LPC in some systems. Recently, LPC-induced mobilization of G2A located in the secretary vesicles to plasma membrane was proposed as a mechanism for LPC modulation of G2A (Frasch *et al.,* 2007).

Many actions of LPC were blocked or inhibited by PAFR antagonists (Ogita *et al.,* 1997; Gómez-Muñoz *et al.,* 1999; Coutant *et al.,* 2002). However, no studies were conducted so far regarding the precise molecular interactions between LPC and PAF receptor signaling. It is to be noted that all such data were from antagonist studies, and no genetic data are available on the involvement of PAFR in LPC actions. Interestingly, WEB 2170, one of the most widely used PAFR antagonist, partially inhibited LPC-induced priming, but WEB 2347, a highly selective PAFR antagonist had no effect (Silliman *et al.*, 2003). Furthermore, WEB 2170 also inhibited leukotriene B_4 (LTB_4)- and oleoyl-acetylglycerol (OAG)-induce priming of fMLP-activated respiratory burst (Silliman *et al.,* 2003). Immune cells with knock-downed PAFR would be useful to prove more definitely the interaction of LPC with PAFR signaling.

G proteins

Studies indicate the involvement of Gi, Gs, Gq and G12/13 in LPC actions in immune cells. Many actions of LPC are blocked or inhibited by PTX, a Gi blocker (Huang *et al.,* 1999; Jing *et al.,* 2000; Coutant *et al.,* 2002; Oestvang *et al.,* 2003; Silliman *et al.,* 2003; Legradi *et al.,* 2004; Yun *et al.,* 2004; Yang *et al.*, 2005). LPC increased cAMP levels in monocytes (Engelmann *et al.*, 1999), THP-1 cells (Yuan *et al.*, 1996), and neutrophils (Lin *et al.,* 2005), indicating the involvement of Gs protein in LPC action in immune cells. Notably, G2A-dependent chemotaxis of macrophages is dependent on Gq and G12/13 (Yang *et al.,* 2005).

In vivo immuno-modulatory actions of LPC

Compared to *in vitro* data, *in vivo* data of immuno-modulatory actions of LPC is relatively scant. Treatment of mice with LPC induces enhanced phagocytic activity of macrophages (Ngwenya *et al.*, 1985). Vitamin D_3-binding protein was reported to play an important role in this response (Yamamoto *et al.,* 1996). Micro-injection of LPC into the spinal cord induces increased recruitment of immune cells, including T cells, neutrophils, and Mac-1+ monocyte (Ousman *et al.,* 2000). Intracutaneous injection of LPC in healthy volunteers similarly elicited acute inflammation with the accumulation of T lymphocytes, monocytes and neutrophils (Ryborg *et al.,* 2000).

Systemic administration of LPC (C18:0; 10 mg/kg s.c., four times every 12 h) protected mice against experimental sepsis-induced lethality, enhanced bacterial clearance and inhibited experimental sepsis-induced increase in TNF-α and IL-1β, and enhanced IFN-γ and IL-2 levels (Yan *et al.,* 2004). LPC administration inhibited LPS- or cecal ligation and puncture (CLP)-induced increase in plasma HMGB1 levels in mice (Chen *et al.,* 2005). Furthermore, LPC administration inhibited LPS- or peptidoglycan/lipoteichoic acid-induced multiple organ damage in rats (Murch *et al.*, 2006).

When injected in hind footpads with protein antigen, LPC was found to act as an adjuvant initiating both humoral and cellular immune responses with an efficiency nearly equal to alum, the standard adjuvant for immunization (Perrin-Cocon *et al.,* 2006).

CONCLUSION

As a major lysophospholipid in the body, LPC having both the pro-inflammatory and anti-inflammatory actions may be more suitable for maintaining homeostasis of the body. Thus, looking at both sides of actions would bring more realistic view of the roles that LPC plays in the biological systems.

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