[Frontiers in Bioscience 9, 915-931, January 1, 2004]

SIGNALING THE BRAIN IN SYSTEMIC INFLAMMATION: THE ROLE OF COMPLEMENT

Clark M. Blatteis, Shuxin Li, Zhonghua Li, Vit Perlik, and Carlos Feleder

Department of Physiology, College of Medicine, University of Tennessee Health Science Center, 894 Union Avenue, Memphis, TN 38163, USA

TABLE OF CONTENTS

1. ABSTRACT

The complement (C) cascade is activated in almost immediate reaction to the appearance in the body of pathogenic microorganims and their products, e.g., bacterial endotoxic lipopolysaccharide (LPS), resulting in the generation of a series of potent bioactive fragments that have critical roles in the innate immune response of the afflicted host, including, potentially, the production of the fever that so characteristically marks bacterial infections. For instance, its derivatives C3a, C3b, iC3b, C5a, and C5b-9 independently induce the production by myeloid and non-myeloid cells of the cytokines interleukin (IL)-1β, IL-6 and tumor necrosis factor-α, and of prostaglandin (PG)E_2, all putative mediators of fever. Therefore, any one of these C components could be involved, centrally or peripherally, in the induction of the febrile response to LPS. Indeed, we have shown that hypocomplementation by cobra venom factor (CVF) dose-dependently attenuates LPS-induced fever in guinea pigs and wild-type (WT) mice, and that C5 gene-ablated mice are unable to develop fever after LPS. In further studies, we found that a specific antagonist to the C5a receptor, $C5aR_1a$, prevents the LPS-induced febrile rise of WT and C3 null mutant mice, implicating C5a as the responsible factor. Various lines of evidence from our laboratory suggest that the macrophages of the liver (Kupffer cells [Kc]) may be the specific target cells of C5a and that the product they release may be PGE_2. PGE_2, in turn, may be the substance that binds to vagal afferents in the liver that convey the pyrogenic message to the brain. Other studies by our group (not included in this review) have separately traced the neural pathway by which this message may be transmitted from the liver to the brain and processed there for action. The purpose of this article is to review the studies that have led us to conclude that C5a, Kc and Kc-generated PGE_2 may be integrally involved in the pathogenesis of LPS fever. If further verified, these results will be important for better understanding how infectious stimuli may trigger the multivariate acute-phase responses generally, and fever particularly, that promptly spring into action to defend the continued well-being of the afflicted host.

2. INTRODUCTION

It is generally considered that fever is caused not by the exogenous infectious noxa (e.g., bacteria and/or their products, such as bacterial endotoxic lipopolysaccharides [LPS]) that initially invade the body, but by endogenous pyrogens, an array of factors belonging to the class of

peptide immunomediators called cytokines, released in response to the entry of the pathogens. Tumor necrosis factor (TNF)α, interleukin (IL)-1β, and IL-6 are believed to be the principal pyrogenic cytokines, elaborated by systemic mononuclear phagocytes activated by the exogenous agents. They are thought to be transported by the bloodstream to the preoptic-anterior hypothalamic area (POA), the brain site of the primary thermoregulatory controller. It is generally thought that the final fever mediator in the POA is prostaglandin (PG)E_2, presumptively induced by these cytokines.

The mechanism that transduces the cytokines' blood-borne message into POA PGE_2 production, however, is controversial (reviewed in 1, 2, 3). Thus, it has been variously proposed that: 1) these cytokines may be actively transported across the blood-brain barrier (BBB) by cytokine-specific carriers and induce PGE_2 locally in the POA (4, 5); 2) they may enter the brain directly where the BBB is "leaky", i.e., through circumventricular organs, in particular the *organum vasculosum laminae terminalis* (OVLT) (6) which lies on the midline of the medial POA, or, alternatively, they may stimulate relevant receptors on neurons that extend into or near the perivascular space of these organs, with consequent *in situ* production of PGE_2 (7, 8); or 3) the circulating cytokines may not penetrate the brain at all, but rather induce the generation abluminally of PGE_2 by endothelial cells of the POA cerebral microvasculature (9, 10) and/or by perivascular microglia and meningeal macrophages in this region that then diffuses to the neurons (11). Although supported by experimental evidence, these mechanisms have one important shortcoming: all are predicated on the arrival of blood-borne cytokines at their site of action (OVLT, cerebral microvessels, etc.). Although cytokines, in particular IL-6, have indeed been demonstrated in plasma correlatively with the onset of fever induced by LPS administered intraperitoneally (ip), intramuscularly (im), and subcutaneously (sc; into an air pouch) (12), they are not detectable concurrently with the induction of fever provoked by iv injected LPS. For instance, LPS at a dose of 2 μg/kg in guinea pigs (13) and 5 μg/kg in rats (14) significantly raises core temperature (T_c) within 10–12 min after its iv injection, whereas TNFα, the first of the cytokines to appear in the blood of similarly LPS-challenged conscious guinea pigs (15) and rats (16), is not detectable until 30 min later. This, however, is not surprising since these cytokines are not expressed constitutively in mononuclear phagocytes, but rather are transcribed, translated, and secreted by these cells in response to the infectious stimulus. While this interval accords with the onset latencies of fevers induced by low to moderate doses of LPS administered ip (~30-60 min), it is, however, too long relative to those after iv LPS. Moreover, these onset latencies become shorter as the ip dose increases so that, at higher doses, they can be as short as those after iv LPS (17). Hence, if they are not yet present in the blood, it would seem improbable that circulating cytokines could provide the signals for the very prompt induction of fever after iv LPS or after high doses of ip LPS. Finally in this context, LPS fever is initiated before the synthases that generate PGE_2, cyclooxygenase (COX)-2 and microsomal prostaglandin E synthase-1 (mPGES-1), are upregulated in the POA (18).

But on the other hand, the levels of pyrogenic cytokines could rise to levels sufficient to excite appropriate sensory neurons in the vicinity of the cells that produce them, if they existed there, well before these mediators would be detectable in the general circulation. Because circulating LPS is cleared primarily by hepatic macrophages (Kupffer cells [Kc]) and because Kc constitute the single largest population of macrophages in the body, they are generally taken to be the major source of pyrogenic cytokines induced by LPS. In 1996, we proposed (19) that the rapidity of the febrile response to iv LPS implied a neural rather than a humoral pathway of communication between peripheral cytokines and the POA, i.e., that sensory nerves originating in the liver could convey these pyrogenic messages to the brain. In support, it had been shown earlier that the injection of IL-1β into the hepatic portal vein increases the electrical activity of the vagus (20) and that the expression of c-*fos* in the nucleus of the solitary tract (the primary projection area of the vagus nerves) is enhanced after iv IL-1β (21). Correspondently, we (19) and several other groups (22, 23, 24) showed that bilateral truncal subdiaphragmatic vagotomy and, more particularly, selective hepatic vagotomy (25), inhibit the febrile responses of guinea pigs and rats to iv LPS. It was further demonstrated that paraganglia on hepatic branches of the vagus bind IL-1 receptor antagonist (26), and that abdominal sensory nerve desensitization by ip administration of low doses of capsaicin inhibits iv LPS-induced fever in rats (27). Taken together, these data, and others not described, thus substantiated the thesis that circulating LPS arriving in the liver may activate Kc to produce and release TNFα, IL-1β and IL-6, in conformity with the prevailing view, and that their local accumulation may stimulate cognate receptors on hepatic vagal afferents that then would rapidly convey their messages to the POA well before they were detectable in the general circulation (1, 28).

There is, however, a weakness in this concept also: although the neural route is more rapid than the blood-borne route of communication, its activation is nevertheless temporally contingent on the presence of these cytokines in the liver sinusoids. But cytokines are not expressed constitutively by Kc; rather, they are transcribed and translated upon LPS stimulation, a process longer than the short latency of the febrile response to iv LPS (e.g., TNFα, as already mentioned, is not detectable in blood until 30 min after the iv injection of LPS; *in vitro*, its half-maximal induction requires a minimum 20 min of contact with LPS [29]). Moreover, by virtue of their continuous exposure to low-grade, gut-derived LPS, Kc are tolerant to it and, consequently, their production of cytokines is downregulated as compared to that of other macrophages *in vivo* (30, 31; see also later). Hence, it would seem unlikely that Kc-generated cytokines could provide the signals that rapidly initiate the febrile response to iv LPS at any dose or to ip LPS at doses higher than 16 μg/kg (17). We speculated therefore that, alternatively, the pyrogenic effect of LPS may be mediated by a factor elaborated by Kc earlier than cytokines, i.e., in almost immediate reaction to the presence of LPS. The question then became: what is this factor and how is it elicited?

We hypothesized that PGE_2 could be this candidate factor. It is synthesized by all macrophages, including Kc, in response to LPS, its level rises quickly in plasma after an LPS challenge correlatively with the febrile rise, and its receptors are widely distributed on sensory neurons, including hepatic and abdominal vagal afferents (32, 33). It could therefore

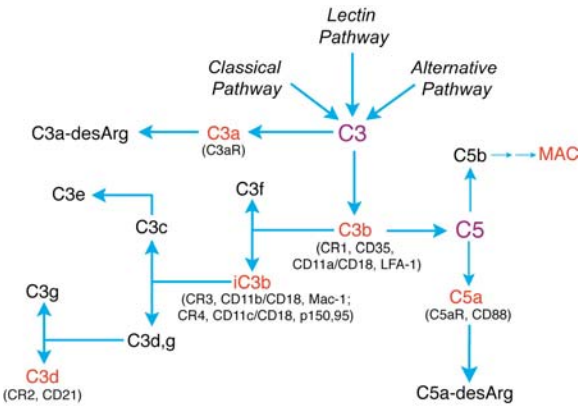

Figure 1. Abbreviated schematic of the complement cascade. C3 and C5 are the pivotal peptides in the cascade; the components labeled in red are the peptides that were presumed to play roles in the febrile response to LPS and were therefore tested. The labels between parentheses below these indicate their respective receptors; several are identified by more than one name. The enzymes involved at different stages of the cascade are not shown.

function as the direct activator of these terminals. Again, however, there is a difficulty: LPS is actually a weak trigger of arachidonic acid (AA) release. Indeed, the activation by LPS of group IV cytosolic phospholipase A_2 ($cPLA_2$, the isoform of the enzyme that initiates the cascade leading to the production by macrophages of PGE_2 from membrane phospholipids) is significantly delayed *in vitro* (34, 35) compared with the prompt elevation of plasma PGE_2 *in vivo* after iv LPS administration (36). Moreover, the increased synthesis of PGE_2 by LPS-stimulated macrophages is selectively catalyzed by COX-2, the transcription and translation of which require at least 1 h *in vitro* (37, 38, 39, 40), i.e., its production lags the febrile response. It would seem improbable, therefore, that LPS could directly account for the rapid appearance of PGE_2 in plasma, and hence also for the activation by the latter of hepatic vagal afferents. The rapid elevation of PGE_2 after iv LPS, we presumed, would consequently have to be mediated not by COX-2, but by COX-1 (or its alter ego, COX-3 [41]) activated by a very rapidly evoked agonist.

Since an important early event in inflammation is the activation of the complement (C) system, a complex series of proteolytic interactions which result in the sequential production of various C proteins that can activate macrophages, we proposed that such an agonist could be a C component (1, 42). Indeed, the iv administration of LPS triggers within 2 min the C cascade via the alternative pathway, resulting in the production in blood of C4a, C3a and C5a, and of surface-bound and fluid-phase C3b and iC3b (reviewed in 43). Kc express the receptors for these C components, and it has been demonstrated *in vitro* that the production of PGE_2 by Kc is initiated within minutes after the addition of, in particular, C3a and C5a, whereas C depletion limits this release (44, 45, 46, 47, 48, 49; see also later). Hence, we hypothesized that the peripheral fever trigger could be PGE_2 released by Kc stimulated by LPS-activated C components and binding to EP receptors on sensory nerves (EP_3 are expressed on sensory neurons and EP_3 knockouts do not develop fever after iv LPS [50]; EP_1 and EP_4 receptors have also been implicated in the febrile response [51, 52, 53, 54]). To test this hypothesis, we hypocomplemented conscious guinea pigs with cobra venom factor (CVF). CVF causes the continuous, uncontrolled activation of the alternative pathway of C, so that the generation of all components subsequent to C3 is dose-dependently reduced because of the gradual depletion of the substrate from which they are produced (55, 56), leading to a proportionately long-lasting (days) hypocomplementemia (57). We found that the fevers caused in these animals by ip LPS were attenuated in direct correlation with the amount of C reduction (57). CVF *per se* injected iv caused an immediate, but transient fall in T_c; C components *per se*, therefore, seemed unlikely to be the pyrogenic factors that directly stimulate the vagal receptors.

Based on this background, we then conducted additional experiments to better substantiate and more fully characterize the involvement of C in the febrile response to LPS (57, 58, 59, 60), and to establish with reasonable confidence that the postulated sequence C, Kc and Kc-generated PGE_2 may indeed be integral to this response (17). The purpose of this article, therefore, is to review our findings to date and to put them into perspective in the context of peripheral LPS sensing and signaling. To our best knowledge, no study has as yet been reported by other groups on this possible pathway of fever induction. We apologize to our readers for what, in the absence of comparable data, is therefore an emphasis on our own work.

We begin with a brief introduction to the C system for those less familiar with its lexicon, focusing on the biology of the components pertinent to the present topic, then describe and interpret our observations, and conclude with a summary and a perspective. A portion of these results has been reviewed previously (17).

3. THE COMPLEMENT SYSTEM

3.1. Overview

The C system is a central component of innate immunity, helping to defend the host by recognizing pathogenic microorganisms and their products and by initiating inflammatory and immunological responses. It consists of over 30 plasma proteins and glycoproteins (~10% of the total serum proteins) and soluble or membrane-bound receptors that interact with each other. The proteins of the system act in enzyme cascades, each step generating enzymes that act in the following step of the cascade, such that a small, initial stimulus can rapidly result in a large effect. The central step in the cascade is the cleavage of C component 3 (C3) (Figure 1; see above). The smaller fragments thus generated (identified by the suffix "a" following their number) diffuse away from their site of production, attracting leukocytes (chemotaxis) and, on binding to cognate receptors on these cells, activating their effector systems. The larger components (identified by "b) coat microbial and other incompatible surfaces in the immediate vicinity of the activation site (opsonization), allowing their recognition by phagocytic cells expressing their specific receptors, triggering phagocytosis and cell

activation. In addition, C is functionally linked to the activation, regulation and operation of adaptive immunity.

Deficiencies in any one C component are associated with a reduced ability to clear circulating immune complexes or to fight infection, emphasizing the important physiologic role of the C system in host defense. On the other hand, C activation can also be deleterious. For example, large-scale C activation contributes to the severe morbidity of bacterial sepsis while local C activation may cause tissue injury, e.g., in myocardial infarction, rheumatoid arthritis, possibly Alzheimer's. But on balance, C is more protective than destructive.

3.2. The C proteins

3.2.1. C3: its derivatives and their receptors

C3 is synthesized primarily by hepatocytes and secondarily by monocytes, macrophages, neutrophils, adipocytes, fibroblasts, astrocytes, renal mesangial and epithelial cells, and others. Its production is upregulated by TNFα, IL-1β, IL-6, interferon (IFN)γ, and LPS, among other agonists; in neutrophils, however, its production is downregulated by IL-1β and IFNγ.

The activation of C3 by convertases assembled through any of the three pathways of the C cascade (see later) (or also by various other enzymes, e.g., serum plasmin and kallikrein, neutrophil elastase, and certain bacterial proteases) leads to the cleavage of C3 into the fragments C3a and C3b. C3a is a potent anaphylatoxin, 77 amino acids in length. It binds to its cognate receptor, C3aR, which belongs to the rhodopsin family of seven transmembrane G protein-coupled receptors. It is ca. 50 kD in size and is expressed on platelets, eosinophils, basophils, mast cells, neutrophils, macrophages, and adipocytes. It has also been detected on neurons and astrocytes, bronchial and alveolar epithelial cells, and pulmonary vascular endothelial and smooth muscle cells. C3a thus stimulates the release of histamine and serotonin from platelets, basophils and mast cells, and modulates TNFα and IL-6 synthesis by monocytes and B-lymphocytes. It triggers the contraction of smooth muscle, thus modulating vasomotion, and increases the permeability of small blood vessels. C3a also stimulates the respiratory burst in macrophages, neutrophils, and eosinophils, the synthesis of eosinophil cationic protein, and the adhesion of eosinophils to endothelial cells. Finally, it induces chemotaxis of eosinophils and mast cells, but only weakly that of neutrophils. C3a has a carboxyl-terminal Arg residue that is important for biological activity. Its removal by carboxypeptidase N generates C3a desArg, which is considerably less capable of eliciting inflammatory responses.

C3b and its sequential cleavage products, C3b, iC3b and, eventually, C3d, covalently attach to cell surface carbohydrates and immune aggregates, forming opsonins which serve as ligands for their receptors, CR1, 3, 4, and 2, respectively. CR3 and CR4 consist of two subunits, α (CD11) and β (CD18); the latter a member of the β_2-integrin family. CR3 is expressed on neutrophils, monocytes, eosinophils, NK cells, and B cells of the $CD5^+$ subset; its expression is reduced in macrophages and dendritic cells. CR3 is the primary receptor for iC3b and interacts less well with C3b and C3d. Its ligation stimulates phagocytosis, respiratory burst and degranulation when bound to iC3b-opsonized bacteria. It also induces IL-1β production by monocytes by interacting with soluble CD23. In addition, it serves as an adhesion molecule used by phagocytes for directed migration through vascular endothelium toward inflammatory sites; but it also mediates non-C-dependent adhesive interactions between neutrophils and endothelial cells by interacting with ICAM-1, factor X, and fibrinogen.

CR4 is also a receptor for iC3b. In addition, it binds LPS, fibrinogen. ICAM-1, and denatured peptides. It is highly expressed on macrophages, dendritic cells, neutrophils, monocytes, and activated B cells. Its functions are similar to those of CR3.

CR2 is a glycosylated type I transmembrane protein. It is a receptor for C3d and weakly for iC3b. It is expressed on B-lymphocytes, dendritic cells, thymocytes, basophils, astrocytes, keratinocytes, epithelial cells, and a subpopulation of T-cells. Its ligation on basophils stimulate histamine release. But its principal function is to enhance humoral immune responses of B-cells.

3.2.2. C5: its derivatives and their receptors

C5 is also primarily synthesized by hepatocytes, and secondarily by monocytes, macrophages, type II alveolar cells, lung, spleen and fetal intestine cells. It is not upregulated by IL-1β and IL-6.

C5a is a 74-amino acid fragment of C5 released upon activation by either the classical or alternative pathway C5 convertases (see below). It is very short-lived in plasma, being rapidly inactivated by carboxypeptidase N which removes its terminal Arg, leaving C5a desArg which has a much reduced inflammatory ability. C5a utilizes a 50 kDa receptor that is a G-protein-linked-member of the heptahelical 7-transmembrane spanning protein family; it exists in two isoforms, $C5aR_1$ and $C5aR_2$. Both occur on neutrophils, monocytes, macrophages, dendritic cells, basophils, eosinophils, mast cells, hepatocytes, vascular smooth muscle cells, renal tubular epithelial and mesangial cells, bronchial and alveolar cells, neurons and astrocytes. When activated, they thus mediate macrophage, monocyte, neutrophil, eosinophil, basophil, and T-lymphocyte chemotaxis, O_2^- generation, and degranulation. The consequent expression of adhesion molecules (stored in the granules of neutrophils) at the surface of neutrophils promotes their adhesion to endothelium and, hence, their migration into inflamed tissue, while the release of histamine, leukotrienes, and other vasoactive mediators by mast cells and basophils cause arteriolar vasodilation and increased capillary permeability. In addition, C5a also upregulates the synthesis of hepatic acute-phase reactants, TNFα, IL-1β, IL-6, and PGE_2 (see later).

Binding of C5b to its target initiates the assembly of the C5b-9 MAC (see below). Insertion of MAC into a

Table 1. Febrile responses of various C fragment-deficient ($^{-/-}$) and sufficient ($^{+/+}$) mice to LPS (ip, iv, or icv) and PGE_2 (icv)

Strain	CVF	Ip LPS	Iv LPS	Icv LPS	Icv PGE_2
WT	--	↑	↑	↑	↑
WT	+	→	↑		
$C3^{+/+}$	--	↑	↑		
$C3^{+/+}$	+	→	↑		
$C3^{-/-}$	--	→	↑		
$C5^{+/+}$	--	↑	↑	↑	↑
$C5^{+/+}$	+	→	↑		
$C5^{-/-}$	--	→	→	↑	↑
$CR2^{+/+}$	--	↑			
$CR2^{-/-}$	--	↑			
$CR3^{+/+}$	--	↑	↑		
$CR3^{-/-}$	--	↑	↑		
	C5aRa				
WT	--	↑	↑		
WT	+	→	→		
$C3^{-/-}$	--		↑		
$C3^{-/-}$	+		→		

+ = CVF- or C5aRa-pretreated; -- = vehicle only; ↑ = fever; → = no fever; blank = not tested; N = 6+/group. (PFS controls not shown.)

cell or a bacterial membrane results in cell lysis, due to the formation of ion-permeable channels and/or leaky "pores". On nucleated cells, MAC activates specific signaling pathways through the recruitment of heterotrimetric G proteins resulting in the synthesis and release of inflammatory mediators, e.g., prostaglandins and leukotrienes.

3.3. The activation pathways

C is activated by three mechanisms, the classical, lectin, and alternative pathways (Table 1). All three converge on the activation of C3 and the subsequent formation of a C5 convertase that cleaves C5 (see below), initiating a fourth pathway, the terminal or lytic pathway.

3.3.1. The classical pathway

The classical pathway is initiated by antigen (Ag)-antibody (Ab) interactions, viz., the Fc regions of immunoglobulins (Ig), specifically IgM and certain IgG isotypes, when they bind Ag. This pathway can also be activated via an Ab-independent mechanism by the acute-phase proteins C-reactive protein and serum amyloid P; these bind to the surfaces of many bacterial and viral pathogens as well as to nuclear constituents released by necrotic or apoptotic cells. And it can also be activated by the binding on the membranes of endothelial and certain other cells of natural IgM Abs produced by, e.g., ischemic events. The process begins with the activation by the relevant stimulus of the first component of C, the C1 complex, which consists of C1q, an inhibitor, C1-INH, and two molecules each of the proenzymes, C1r and C1s, two serine proteases. The binding of C1q to its ligand, e.g., Ig, displaces C1-INH from the complex, autoactivating one C1r, which then activates the second C1r. Both C1rs then cleave both C1s to form active C1s. Active C1s, in turn, sequentially cleaves C4, producing C4a and C4b. C4b bound to an activating surface binds C2, which is then cleaved by C1s, yielding C2b and C2a. C2a is released while C2b remains bound to C4b, forming the classical pathway C3 convertase, C4bC2b. It cleaves C3, releasing C3a and C3b. The binding of C3b to the C3 convertase, in turn, forms the classical pathway C5 convertase, C4bC2bC3b, which cleaves C5 into C5a and C5b.

3.3.2. The lectin pathway

The lectin pathway is activated by mannan-binding lectin (MBL). It recognizes repeating microbial surface mannose residues and triggers the activation of MBL-associated serine proteases, MASP-1, -2, and -3 (which are analogous to the C1r/C1s proteases of the classical pathway), cleaving C4 and C2 and leading to the formation of the classical pathway C3 and C5 convertases.

3.3.3. The alternative pathway

The alternative pathway is activated by whole microorganisms and their products, e.g., LPS, teichoic acid, zymosan, and by certain cell surfaces, e.g., erythrocytes, by a process called "tickover". Serum C3, which circulates in a continuously hydrolyzed low-grade state, $C3(H_2O)$, binds factor B, an acute-phase protein upregulated by LPS, TNFα, and IL-1β, forming $C3(H_2O)B$. Factor D, a circulating active serine protease, cleaves it to form a fluid-phase C3 convertase, $C3(H_2O)Bb$, which, in turn, cleaves C3 to yield C3a and C3b. In the presence of an activating surface, C3b binds factor B, forming C3bB, which is further cleaved by factor D, producing the alternative phase C3 convertase, C3bBb, which cleaves C3 into C3a and C3b. This convertase is stabilized by properdin, which acts as an amplifying activator. This enables C3b to bind to it and form the alternative pathway C5 convertase, C3bBbC3b, which cleaves C5 into C5a and C5b. In plasma, C3b is rapidly catabolized by factor I, a serine protease, and a cofactor, factor H; on cell surfaces, the membrane-bound cofactors are C receptor 1 (CR1) and membrane cofactor protein (MCP). Thus, these cofactors and factor B compete

for binding to C3b and thereby regulate, up or down, the activation of C3 by this pathway.

3.3.4. The terminal pathway

The cleavage of C5 by the C5 convertases releases C5a and C5b. The latter binds to C3b in the convertase, forming C3b5b and exposing an acceptor site for C6, resulting in the formation of C5b6. Conformational changes thereafter allow the binding of the subsequent C peptides C7, C8 and C9. A variable number of the latter associate with the C5b678 complex, forming the mature membrane attack complex, MAC (C5b678poly9).

3.4. Decomplementation by cobra venom factor

Cobra venom factor (CVF; usually from *Naja naja kaouthia*) is a toxin commonly used to produce pharmacologically the depletion of C3. It activates the alternative pathway of the C cascade, forming a complex with factor B, CVFBb, which is analogous to C3bBb, the C3 convertase (61). Thus, similarly to it, it cleaves the α chain of C3, thereby increasing the production of C3b, C5, and their other biologically active derivatives. But, whereas C3bBb is very labile ($t_{1/2}$ = 1.5 min at 37 ^{o}C), CVFBb is very stable ($t_{1/2}$ = 7 h) due to its resistance to the control mechanisms that normally limit the activity of C3 convertase (i.e., factors I and H). Consequently, C3 activation continues unabated, drastically reducing C3. Absent the substrate from which they are produced, all the subsequent C components are also ultimately depleted. The amount and duration of the C reduction thus induced are dose-dependent (57).

4. COMPLEMENT AND LPS FEVER

4.1. Characterization of the C-LPS fever interrelationship

To substantiate, initially, our hypothesis that C activation may be pivotal in the induction of fever by iv LPS, we measured the body (core) temperatures (T_c) and the levels of PGE_2 in microdialysis effluents from the POA of conscious guinea pigs over their entire febrile course after iv LPS, with and without reduction of C by the prior iv administration of CVF. CVF pretreatment attenuated the first of the characteristically biphasic T_c rises after iv LPS, inverted the second into a T_c fall, and greatly reduced the usual fever-associated increase in preoptic PGE_2 (58). We concluded, therefore, that C activation may indeed be pivotal in the induction of fever by iv LPS.

Because the dynamics of the T_c rises after iv and ip LPS are different, the minimally effective pyrogenic dose is higher and the latency of fever onset longer after the latter (57), and because other data suggested that the fever caused by ip-administered LPS may be more vulnerable to the antipyretic effect of vagotomy than that caused by iv LPS (62), we investigated, next, whether the C system may also be involved in the febrile response to ip LPS. Thus, 1) we first determined the CVF dose-plasma C level response relation in conscious guinea pigs. 2) We next determined the ip LPS dose-fever response relationship of conscious guinea pigs and, on the basis of the results, chose for our subsequent studies the dose that induced a fever of similar course, albeit with a longer latency, as our standard iv dose (2 μg/kg), *viz*., 8 μg/kg. 3) We then compared the thermal responses of our guinea pigs pretreated either with pyrogen-free saline (PFS) or various doses of CVF to iv or ip PFS or LPS. 4) Finally, we measured the plasma C levels of our guinea pigs at various intervals after iv or ip PFS or LPS. We found that the magnitude and course of the febrile responses to iv LPS were not demonstrably affected by C reduction whereas, on the other hand, the fevers caused by ip LPS were attenuated in direct correlation with the amount of C reduction. The reason for this differential susceptibility to depression by iv and ip administered LPS was not evident from the data. We speculated that it could be due to different functional and biochemical properties of peritoneal and hepatic macrophages because evidence existed that the activation of macrophages by LPS for synthetic responses proceeds by several pathways. Thus, while membrane (m) CD14 is the predominant LPS receptor on macrophages and soluble (s) CD14 that on cells lacking mCD14, e.g., endothelial cells, their activation requires that LPS complexes to LPS-binding protein (LBP), which is present in normal plasma but scarce in plasma-free peritoneal and other fluids (63, 64). Indeed, it had been shown that only minimal amounts of LPS-induced cytokines and PGE_2 are released by macrophages in the absence of LBP. If, then, LPS-CD14 interactions were not favored in the peritoneal fluid because of the lack of LBP and yet fever developed after ip LPS, we surmised that a different signaling mechanism might activate peritoneal macrophages, one that, according to these data, might be critically dependent on C. Such a process could be provided by CR3 and CR4, which had been shown to function as LPS transmembrane receptors that activate macrophages (65, 66), including in the presence of anti-CD14 monoclonal antibodies (67, 68); as discussed earlier, these receptors recognize iC3b-opsonized LPS particles. Binding sites for LPS and for iC3b co-exist in the same molecule, thus co-mediating the attachment of iC3b-coated LPS to macrophages (69, 70) and inducing IL-1β and TNFα synthesis (71, 72). Hence, we speculated that LPS and iC3b-opsonized LPS, in tandem, plus C3a and C5a, also induced by LPS, may all be required to activate peritoneal macrophages through these signaling pathways. A decrease in C proteins for opsonization of LPS as well as *per se* may be expected, therefore, to concentration-dependently impede the ability of peritoneal macrophages to respond to LPS. However, rational as it seemed then, this interpretation was not substantiated by subsequent data (see later).

A question that arose from the preceding studies was whether the mediatory role of C is specifically limited to the febrile response to LPS or is manifested generally in fevers caused by all pyrogens. To answer this question, we repeated our LPS studies, but using different pyrogens, *viz.,* muramyl dipeptide (MDP, a synthetic Gram-positive bacterial cell-wall analog) and polyriboinosinic:polyribocytidylic acid (poly I:C, a synthetic viral double-stranded RNA analog), i.e., factors that reportedly induce fever through a cytokine- and PGE_2-mediated process similar to that of LPS (73, 74, 75, 76). Our results (59) indicated, however, that C is not involved

in the febrile responses to these agents. Their genesis, therefore, presumably proceeds by mechanisms different than that utilized by LPS. MDP, for one, may penetrate the brain directly and stimulate IL-1βand/or PGE_2 production locally (77). But we have not yet investigated what mechanisms might be operating in their cases.

Further in this context, we examined whether the C system might be involved in the febrile response to zymosan, a cell wall product of baker's yeast which activates C by the alternative pathway even more strongly than LPS and is reported to be pyrogenic via the induction of cytokines and PGE_2, similarly to LPS (78). Indeed, we found (79) that zymosan injected at 0.5 mg/kg iv into conscious guinea pigs induced 1 ^oC fevers, but at 25 mg/kg iv, it produced quick-onset, 1 ^oC T_c falls. A second injection at the higher dose 3.5 h later yielded smaller and briefer T_c falls. We attributed the smaller response to the second injection to the reduction of C produced by the first injection, analogous to the effect of consecutive iv injections of CVF (58). CVF pretreatment, on the other hand, converted the T_c fall after the first high-dose zymosan injection into a T_c rise. These results thus suggest that zymosan may be inherently pyrogenic, but that this effect may be manifested only when the dose of zymosan is too small to activate C or when C has been reduced by prior activation of the C cascade. Hence, C would not seem to be a mediator of the febrile response to zymosan, but rather of its cryogenic effect. We have not studied this phenomenon beyond this point (79).

4.2. Identification of the C component mediating the febrile response to LPS

The next question, therefore, became: which among the various, possible C components mentioned above is, in fact, involved in the mediation of the febrile response to LPS? Because of the lack of suitable, selective drugs administrable to conscious guinea pigs, but, on the other hand, their availability for use in mice, our experiments designed to address this question were conducted in conscious mice, using null mutants and wild-type (WT) littermates or strain-matched mice for controls.

To verify initially that C is involved in the febrile response of mice to LPS as it is in that of guinea pigs, we repeated our earlier studies in guinea pigs, i.e., we depleted C with CVF and measured the T_c responses of WT mice to LPS (1 μg/mouse, ip or 0.25 μg/mouse, iv). Like guinea pigs, these animals responded to iv LPS with similar fevers as their CVF vehicle-pretreated controls, but did not develop fever after ip LPS (81). These results thus confirmed the involvement of C in the fever production of mice after ip but not iv LPS, and established the suitability of this species for these studies. Following the same protocol, we therefore challenged C3 and C5 gene-ablated and C3- and C5-sufficient mice hypocomplemented with CVF with ip LPS. None of these animals developed fever in response to this challenge (80). On the assumption, therefore, that C3-deficient mice lack C5 whereas C5-deficient mice express C3, we concluded that C5 is the critical mediator of the febrile response to ip LPS; but we did not fully rule out that C3 may also play a role (81). To distinguish, therefore, between C3 and C5 and subsequently, as warranted, between C3a and C3b and its derivatives, and between C5a and C5b-downstream components, and also to elucidate the observed, apparently greater susceptibility of ip than of iv LPS-induced fever to C reduction, we repeated the previous experiments, injecting LPS iv. All the mice excepting the C5 knockouts developed T_c rises following this challenge (82). In subsequent studies, we found (80, 82) that CR2- and CR3-deficient mice responded normally to ip and iv LPS, further implicating C5 but not C3 (through C3d and iC3b, respectively), as the component presumptively critically involved in the febrile response. Indeed, in a follow-up experiment, a specific antagonist of $C5aR_1$, $C5aR_1a$, prevented the febrile rises of WT mice to both ip and iv LPS and of $C3^{-/-}$ mice to iv LPS, indicating that C5a may indeed be the crucial mediator of this response (83). Finally in this regard, to exclude the possibility that $C5^{-/-}$ mice may be generally impaired and cannot develop fever at all and in view of the now recognized role of the C system as an integral part of the natural innate immune system of the brain (84), we administered PGE_2 and LPS icv to C5 knockouts. They also developed normal fevers, indicating that their central thermoregulatory mechanism is not impaired and that the fever-mediating site of action of C5a is upstream from the brain. i.e., peripheral rather than central (85). These results are summarized in Table 1.

The finding that the $C5^{-/-}$ mice did not develop fever in response to both ip and iv LPS would suggest that the greater susceptibility to the absence of C of the febrile response to ip than to iv LPS observed earlier may not exist. We account for the apparently discrepant responses that we saw earlier as follows. 1) In these and our previous studies, we used CVF to decomplement our guinea pigs and mice. However, we found in our earlier studies in guinea pigs that decomplementation was never complete, even at the highest CVF dose used (57). Thus, it is probable that some C5 remained that could have mediated the observed febrile responses of the CVF-treated guinea pigs and mice to iv LPS. 2) It has been shown that small amounts of C5a may be generated directly by a complexed C4/2 convertase capable of activating C5 directly, bypassing C3 (86, 87, 88). The presence in plasma of a small amount of C5 derived via this pathway, therefore, could have been sufficient to enable the febrile responses of the CVF-hypocomplementemic guinea pigs and mice and of the C3 knockouts to iv injected LPS. 3) We have shown recently (see below) that the onset of LPS-induced fever is temporally related, irrespective of its route of administration (iv or ip), to the appearance of LPS in liver and its uptake by Kc (89, 90), suggesting that C may be activated by contact with LPS in the liver rather than in the bloodstream. This, in turn, infers that C5 may be generated independently of plasma-derived C, i.e., by extravascular components of the liver, e.g., by Kc. Indeed, as reviewed earlier, synthesis of C5 by human and mouse macrophages has been described (91, 92, 93, 94, 95). Macrophages also produce C5-cleaving serine proteases that can generate C5a (96, 97, 98, 99), and Kc express C5aR (100, 101). Thus, it is possible that C5a is locally generated by LPS-activated Kc from its autogenously produced C5 and functions as an

autocrine activator of Kc. Such a discrete, focal localization of C5a production could also account for the observed febrile responses of our C-deficient guinea pigs and mice to iv LPS.

5. THE CENTRALITY OF THE LIVER IN LPS-INDUCED FEVER

5.1. Fever and the liver's Kupffer cells

It may be presumed that the route of LPS administration (i.e., the mode of entry of infectious pathogens) determines the mononuclear phagocyte population predominantly activated, at least initially, to produce cytokines. Since liver is the main clearance organ for circulating LPS (102, 103, 104), and Kc, which account from 80% to 90% of the total population of fixed tissue macrophages in the body (105, 106), are believed to be responsible for this clearance, Kc, as suggested in the Introduction, may be pivotally involved in the production of cytokines and PGE_2, and hence also in the fever induced by blood-borne LPS. Since, moreover, Kc express the receptors for certain C proteins, the Kc production of cytokines and $PGE_2$is initiated after their addition, and both the febrile and preoptic PGE_2rises after iv LPS are abolished by vagal transection, the postulated critical involvement of Kc in the initiation of the febrile response to iv LPS administration seems highly plausible.

To investigate, therefore, the role of Kc in the initiation of the febrile response of conscious guinea pigs to iv LPS, we hypothesized that their selective depletion should eliminate them as a source of pyrogenic cytokines; if fever were thereby prevented, their role would indeed be critical. To deplete Kc, we used gadolinium chloride hexahydrate ($GdCl_3$), a lanthanide phagocytosed by macrophages, which leads to their disappearance within 12 h. Repopulation of macrophages starts 2 d later in spleen and lungs, and after 4 d in liver (107). Consequently, we conducted our experiments 3 d after $GdCl_3$ administration, when only the Kc should be absent. In contrast to the characteristically biphasic fevers and increases in preoptic PGE_2 levels exhibited by the guinea pigs that received iv LPS, guinea pigs that were pretreated with $GdCl_3$ exhibited a T_c fall and no increase in their preoptic PGE_2 levels (60). We concluded, therefore, that Kc are indeed pivotal in the induction of fever by iv LPS and that a substance generated by these cells in almost immediate reaction to the presence of LPS and/or C may transmit pyrogenic signals via hepatic vagal afferents to the POA. To verify subsequently whether Kc could indeed bind LPS within 15 min *in vivo*, we injected fluorescein isothiocyanate (FITC)-labeled LPS iv into conscious guinea pigs (58). Using confocal laser scanning microscopy, we examined their livers for accumulation of the label 15 and 60 min later. Granular fluorescent patches were detectable on or in Kc in the liver sinusoids 15 min and in hepatocytes 60 min after its administration; only normal autofluorescence was apparent in control animals that received fluorescein salt or LPS alone. These data supported our proposition, therefore, that LPS injected iv may induce fever within 15 min via a Kc-dependent process.

Ip LPS-induced fever, on the other hand, is thought to be initiated by peritoneal macrophages (PM) capturing the LPS and consequently releasing cytokines that enter the circulation and ultimately act on the same distal targets as those stimulated by Kc-generated cytokines (1, 2, 3). Alternatively, it has been suggested that these cytokines may directly stimulate local abdominal sensors that convey the pyrogenic signals neurally to the POA (26). In contradiction to ip generated cytokines as the mediators of ip LPS-induced fever, however, other, concurrent acute-phase responses to ip LPS, viz., increases in circulating IL-6, adrenocorticotropic hormone and corticosterone, correlate with circulating LPS levels but not with LPS or cytokine levels within the peritoneal cavity (108, 109). These observations would suggest that the febrile response could also be triggered by LPS that has passed from the peritoneum into the bloodstream rather than by cytokines released by PM acting locally or remotely. Consequently, the initiation of the pyrogenic response to ip LPS, like that to iv LPS, could depend on the accumulation of LPS to a threshold level in a site where its passage is virtually assured and its sequestration possible, viz., the liver's Kc, and the consequent generation of cytokines or PGE_2 by these cells.

To test this hypothesis, we assessed the relative contributions of hepatic and other mononuclear phagocytes (in blood, peritoneum, lungs, kidneys, spleen, mesenteric lymph nodes, and brain) to the febrile response of conscious guinea pigs to LPS (as analogized by FITC-LPS) injected iv and ip. Thus, we measured the courses over 2 h of the uptake and the associated T_c changes induced by the labeled LPS administered at various doses, from pyrogenic to toxic, and by the two routes; unlabeled LPS was used to evaluate the pyrogenic equivalencies of FITC-LPS. $GdCl_3$ was used to verify the role of Kc in the observed febrile responses. At all time points evaluated, iv FITC-LPS appeared in circulating leukocytes and Kc; its density was proportional to dose (Figure 2A). At all doses tested, the density of iv FITC-LPS labeling decreased from its peak 15 min after injection at a rate commensurate with its dose. Ip FITC-LPS was also detectable dose- and time-dependently in PM, but it appeared later and accumulated more slowly in Kc, in inverse proportion to dose (Figure 3); compared with iv FITC-LPS, its maximal presence was always lower in density (Figure 2B). No labeling was found at any time in brain and kidneys following any dose of iv or ip FITC-LPS; splenic and lymph node uptakes could not be evaluated due to high tissue autofluorescence. Pretreatment with $GdCl_3$ 3 days before LPS injection attenuated the early phase of the febrile response to iv and ip injected FITC-LPS and reduced significantly the density of its label in the liver and leukocytes. The onset of the T_c rises, i.e., the early phase of the febrile response, was correlated temporally with the appearance of FITC-LPS in liver and leukocytes when it was given iv, and in liver only when it was given ip. For example, the appearance of fluorescence in Kc was coincident with the first elevation of T_c 15 min after the iv injection of a pyrogenic dose of FITC-LPS, i.e., significantly in advance of the time when new cytokines would be expected to be generated and released

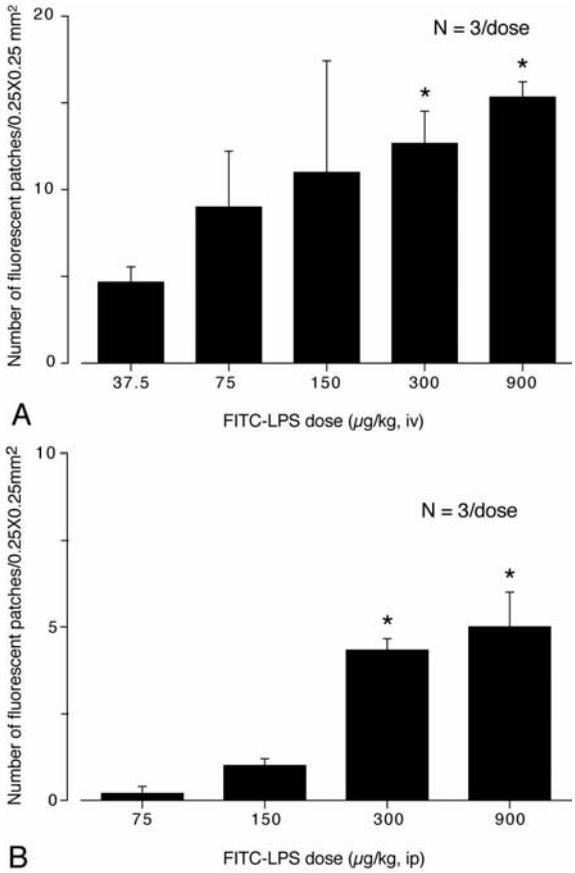

Figure 2. Distribution of iv (A) and ip (B) injected FITC-LPS in livers 15 min after its administration to conscious guinea pigs in relation to dose. The number of fluorescent patches was counted over a 0.25 x 0.25 mm^2 area of liver tissue and expressed as the average value of 5 different areas per animal. N = number of animals per dose. The values are means ± S.E. All the values are significantly different from 0 (no FITC-LPS; *: significantly different ($P<0.05$) from 37.5 μg (iv) or 75 (ip) FITC-LPS/kg, respectively.

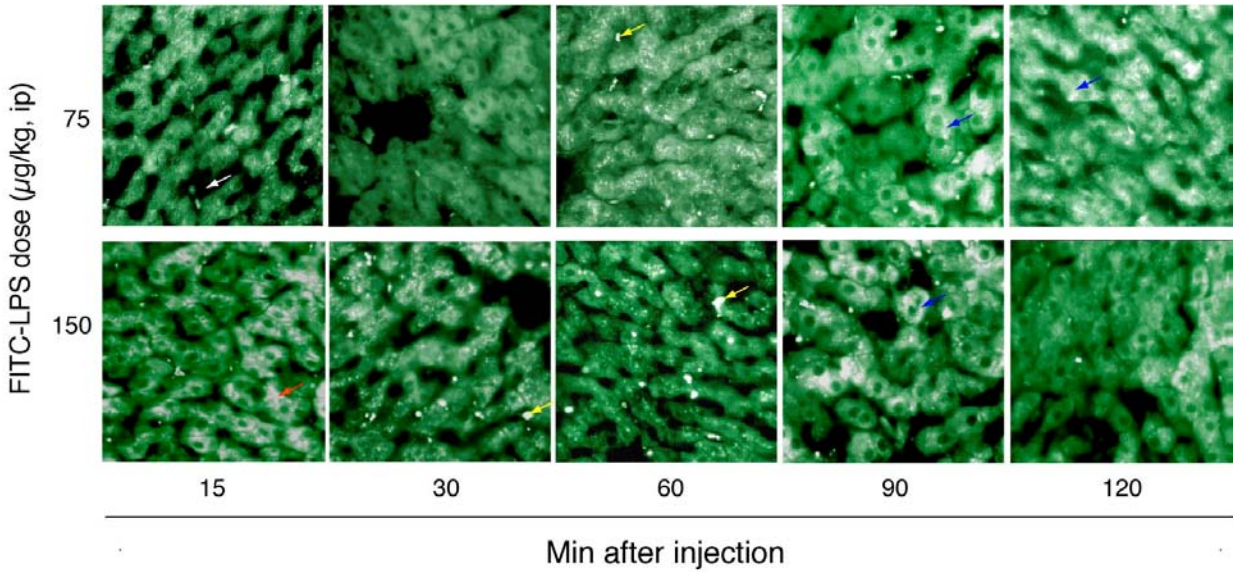

Figure 3. Distribution of ip injected FITC-LPS in livers of guinea pigs at doses of 75 and 150 μg/kg at different times after administration to the conscious animals. White arrow: liver sinusoids; red arrow: autofluorescence; yellow arrows: labeled Kupffer cells; blue arrows: labeled hepatocytes. Magnification: 200 x.

into the circulation (see also later). A similar, direct relationship between Kc labeling and T_c rises was evident after ip FITC-LPS, although later in time than after iv FITC-LPS. The continuation of the febrile course beyond its initial rise, however, was unconnected to the presence of FITC-LPS in Kc, irrespective of its route of administration.

These data would thus support our hypothesis that iv and ip LPS may share a common mechanism to initiate fever and that Kc (and leukocytes) may play a central role in this mechanism. However, other cell types in the liver that also react to C, e.g., hepatic stellate cells, sinusoidal endothelial cells, mast cells, recruited neutrophils, and liver-associated lymphocytes, cannot yet be ruled out as also having possible roles in fever initiation. The contributions to fever induction by other macrophages within the vasculature, however, would appear inconsequential since their uptake of FITC-LPS did not correlate temporally with the observed T_c changes. Indeed, the lack of correlation between the fluorescent labeling of the PM and the T_c rises after ip FITC-LPS is contradictory to some previous papers (26), but is consistent with the transport of LPS from the peritoneal cavity into the circulation by the lymphogenous route (110, 111, 112), as we had posited. Taken together, these results therefore are congruous with our hypothesis that a factor rapidly induced by LPS but different than cytokines may be generated by C-activated Kc and provide the pyrogenic signal that is transmitted to the POA. The subsequent course of the fevers induced by both iv and ip LPS, on the other hand, are likely sustained by meanwhile released cytokines acting on their various other, proposed targets (e.g., OVLT, cerebral microvessels, etc.).

The involvement of the liver in the pathogenesis of fever is not a new discovery. It was first suggested a long time ago (113) and has since been supported by a variety of findings (27, 114, 115). In this regard, therefore, the present results only confirm, by another means, that previous notion. Their novelty, however, lies in their implication that, in conformity with our hypothesis, cytokines may not be necessary for the induction of the febrile response to LPS. To substantiate this interpretation, we sought, first, to verify by further means the central role of the liver in LPS-induced fever, and, second, to determine the correlation between the uptake of LPS by the liver and the circulating levels of TNFα, IL-1β, IL-6, and PGE_2 during the courses of the febrile responses to various doses of ip and iv LPS in conscious guinea pigs.

5.3. Fever, Kupffer cells, and the spleen

To address the first proposition, because splenic macrophages also contribute importantly to the intravascular clearance of LPS (102) and, together with lymphocytes, produce abundant IL-1β (116) and liberate factors into the portal circulation that modulate Kc function (117), we evaluated the effect of splenectomy on the febrile response of conscious guinea pigs to iv and ip LPS 7 and 30 days after surgery. FITC-LPS uptake by Kc, PM, lungs, kidneys, mesenteric lymph nodes, leukocytes, and brain was assessed correlatively at intervals during 1 h after the LPS challenge (118). LPS at 0.05 μg/kg iv 7 days post-surgery did not evoke fever in sham-operated animals but caused a 1.2 oC Tc rise in the splenectomized group; 2 μg/kg iv induced a 1.8 oC greater Tc rise in the splenectomized than in the control guinea pigs. LPS at 2 and 8 μg/kg ip induced 1.4 and 1.8.oC higher fevers, respectively, in the splenectomized than the sham-operated animals. The febrile responses of the asplenic animals were similarly increased 30 days after the surgery. At 7 days, FITC-LPS was detected in the liver of the controls 15 min after its iv administration; its density was reduced at 30 min and virtually null at 60 min. In contrast, the labeling was significantly denser in the splenectomized animals and continued unchanged at all three time points; this effect was still present 30 days after surgery. Similar results were obtained at 60 min after ip FITC-LPS. The distribution and density of FITC-LPS fluorescence in lungs, kidneys, leukocytes, brain (delivered iv or ip), and PM and lymph nodes (ip only) of the sham and splenectomized guinea pigs 7 and 30 days post-surgery were not different. GdCl3 pretreatment of the splenectomized animals significantly reduced both their febrile response to LPS (8 μg/kg ip) and their Kc uptake of FITC-LPS 7 days post-surgery.

This study thus demonstrated that splenectomy results in significantly higher fevers in response to both iv and ip LPS and that this effect is evident not only immediately after the operation, but also recurs when LPS is administered 30 days after surgery. Indeed, infected asplenic patients also generally exhibit recurrent, higher fevers than infected eusplenic patients (119). These results further showed that these intensified fevers are associated with a significantly larger uptake of (FITC-) LPS by Kc. Since the elimination of Kc by GdCl3 attenuated both the febrile responses and the intensity of the labeling in the livers of both the sham and the splenectomized animals and in view of the absence of differences in the density of fluorescence in other mononuclear phagocytes, a close link between LPS-induced fever and the liver is again implied. Furthermore, these data indicate that the spleen may exert a modulating influence, presumably inhibitory, on the uptake of LPS by the liver; i.e., the observed increase in the uptake of LPS probably does not represent a simple effort by the Kc to counterbalance the absence of splenic macrophages (i.e., no capture of LPS by splenic macrophages → more circulating LPS → greater LPS uptake by Kc → enhanced production of pyrogenic mediator[s]) because, were this the case, one might expect that the larger output of endogenous pyrogens by the liver would serve to quantitatively replace that missing due to the removal of the spleen and the magnitude of the febrile response would consequently be unaffected rather than enhanced, as observed. This is a new observation.

To determine whether the enhancing effect of splenectomy on the avidity of Kc for LPS may indeed be specifically dependent on a modulator released by the spleen into the portal circulation, we ligated the splenic veins of guinea pigs, and 7 and 30 days later again assessed continuously over 6 h the conscious animals' febrile responses to LPS (2 μg/kg ip) and the uptake by their livers of FITC-LPS (75 μg/kg ip) 60 min after administration. Splenic vein ligation

caused enhanced fevers and FITC-LPS uptake at 7 days, similarly to splenectomy. At 30 days post-surgery, these effects were considerably moderated: however, new collateral veins were now abundantly evident around the ligated splenic vein. These data thus support the view that the spleen may modulate LPS uptake by Kc, perhaps by contributing a factor that downregulates their affinity for LPS.

5.3. Fever, Kupffer cells, and neutrophils

Neutrophils are another putatively important source of endogenous pyrogens (120, 121). Moreover, as described earlier, the onset of fever is linked not only to the appearance of LPS in Kc, but also in circulating leukocytes. On the other hand, "febrile neutropenia" is a well known clinical syndrome of infected immunocompromised patients treated with neutrophil-depressing chemotherapeutic agents (122), suggesting that the contribution of neutrophils, when absent, to fever production may be replaced by that of other phagocytes, e.g., Kc. To test this hypothesis and also to further substantiate the implied centrality of Kc as contrasted to other mononuclear phagocytes in the febrile response to LPS, we induced neutropenia in guinea pigs by iv treatment with vinblastine, and 4 d later, as above, assessed continuously over 6 h the febrile responses of the conscious animals to iv LPS and the uptake by their livers of iv FITC-LPS 60 min after injection. As expected, the fevers of the neutropenic guinea pigs were significantly higher and more prolonged than those of their vehicle-treated counterparts. Their Kc uptake of FITC-LPS was also significantly augmented as compared to that of their controls. The induced neutropenia *per se* had no demonstrable effect on either of these variables. How the absence of neutrophils, like that of splenic macrophages, leads to intensified rather than to simply counterbalanced responses to LPS is enigmatic. But be that as it may, these findings further support the central role of the liver in LPS-induced fever.

5.4. Fever, Kupffer cells, and complement

Finally in this context, in view of the antipyretic effect of decomplementation described earlier, we examined whether the absence of C might impair the uptake of FITC-LPS by the liver and thereby account for the blockade of the febrile response. To this end, we pretreated guinea pigs, as before, with two doses of CVF, and 21 h later administered ip to the conscious animals two doses of FITC-LPS. Kc labeling was measured 60 min later. As earlier, the density of the labels was dose-related. No difference was found between the PFS- and CVF-pretreated animals, indicating that C(5a) does not exert its mediatory function by altering the affinity of Kc for LPS.

5. THE LPS-INDUCED, C-MEDIATED, KC-GENERATED, VAGUS-STIMULATING PYROGENIC MESSENGER

To address the second proposition, as reviewed in the Introduction, the quandary that motivated this entire research is the temporal incongruity that exists between the onset of the febrile response to a bolus iv injection of LPS and the appearance in plasma of pyrogenic cytokines, the presumed drivers of the T_c rise. Indeed, in the experiments reported above, the appearance of fluorescence in Kc was coincident with the first elevation of T_c at 15 min after the iv injection of pyrogenic doses of FITC-LPS, i.e., in advance of the time when new cytokines would be expected to be generated and released into the circulation. Data on the rate of accumulation of cytokines in the peritoneum after a bolus ip LPS challenge are now available (see below), but in anticipation, it should be noted that, in peritonitis, cytokines reportedly tend to remain in the abdominal cavity rather than pass into the bloodstream (123). The observed dose-dependent coincidence between the appearance of labeling in Kc and the T_c rises after ip FITC-LPS, at all doses, would thus imply that the arrival of LPS in the liver rather than its binding to PM and consequent local activation of cytokine production may be the trigger for the initiation of the febrile response to ip LPS. Hence, to further account for the promptness of the febrile response, we posited, as discussed in the Introduction, a vagally rather than a humorally transmitted signaling mechanism to the POA and suggested that the initiation of the febrile response to LPS may be triggered by a non-cytokine mediator very quickly released into the liver sinusoids by an action of LPS on Kc and capable of stimulating local sensory terminals. Our results to-date implicating C5a and the centrality of the liver in the febrile response to LPS, described above, would thus support this concept. Based on various data in the literature, we hypothesized further that PGE_2 may be the possible mediator in this process. It should be noted in this regard that the PGE_2 that may be released in the liver, stimulate local vagal afferents, and circulate in the bloodstream under these conditions is not the one that stimulates thermosensitive neurons in the POA to drive the febrile response: the latter is produced locally in the POA, not transported into it (1, 32, 123, 124).

To test this hypothesis, we conducted three experiments (125). In the first experiment, conscious guinea pigs were injected ip or iv with LPS after pretreatment with CVF or $GdCl_3$. T_c was monitored continuously and LPS, PGE_2, TNFα, IL-1β, and IL-6 in the peritoneal fluid and in plasma were measured at intervals over a 2-h duration after these injections.

The results indicated that ip injected LPS drains very slowly from the peritoneum into the circulation. Thus, peritoneal LPS peaked within 15 min and declined very slowly thereafter; but plasma LPS did not increase significantly until 90 min post-injection. Peritoneal PGE_2 lagged peritoneal LPS by 15 min, but decreased more rapidly; plasma PGE_2 began to rise ca. 60 min after LPS administration. T_c rose concurrently with plasma LPS and PGE_2 levels. CVF pretreatment did not alter the courses of peritoneal LPS and PGE_2 and of plasma LPS, but it significantly attenuated the late rises of both plasma PGE_2 and T_c Thus, the febrile response to LPS was correlated with the rise of plasma, but not peritoneal, PGE_2. TNFα, IL-1β and IL-6 were detectable in the peritoneal fluid 30 min after LPS injection; their appearance in plasma lagged that in the peritoneum by 60 min. CVF pretreatment did not affect these responses. Since C depletion attenuated the

febrile response, yet these cytokines were present in both compartments (as contrasted to the absence of PGE_2 in plasma), fever was not correlated with their presence in either compartment. Pretreatment with $GdCl_3$ affected the responses of these variables in the same way as CVF; i.e., it prevented the T_c and plasma PGE_2 rises without changing the courses of the other variables, thus further reinforcing the central function of Kc in the febrile response to ip LPS. The iv injection of a bolus of LPS resulted, as expected, in its immediate maximal concentration in plasma; its clearance proceeded more rapidly than when it was injected ip. Its appearance in plasma promptly caused T_c to increase correlatively. Pretreatment with CVF or $GdCl_3$ again did not affect the rate of clearance of LPS from plasma, but abrogated the febrile response. The remaining data are incompletely analyzed at this writing.

In the second experiment, we injected directly into the portal vein of anesthetized guinea pigs 2 μg of LPS/kg and measured the plasma levels of LPS, PGE_2, and cytokines in samples collected from the inferior vena cava at close intervals during 1 h following this injection; T_c was not monitored because anesthetized animals cannot develop fever. The purpose of injecting a small dose of LPS directly into the portal vein was to isolate its site of action as much as possible to the liver in order to exclude extra-hepatic effects that could confound the responses observed. Plasma LPS peaked immediately after its injection, then fell rapidly to ~50% of its maximum and stabilized. CVF pretreatment did not change this pattern. Plasma PGE_2 levels increased significantly within 2.5 min following the administration of CVF, peaked in 5 min, then returned to control in 15 min. LPS given 3 h later had no effect on PGE_2 levels until ca. 30 min later, when they tended to increase gradually. In contrast, LPS given to CVF-untreated guinea pigs at the same time induced an immediate PGE_2 rise followed by a quick fall, similarly to those caused by CVF except that PGE_2 returned to only 50% of its initial level; it tended to rise slowly ~25 min later. Thus, the initial, rapid release of hepatic PGE_2 induced by iportal LPS would appear to be entirely due to C. The cytokine data are incompletely analyzed at this writing.

In the third experiment, we determined the levels of PGE_2 and cytokines released by primary Kc and PM stimulated *ex vivo* by C, LPS, IL-1β, or IL-18, and C + these pyrogens. The cells were harvested from WT, COX-$1^{-/-}$ and COX-$2^{-/-}$ mice to determine which COX isoform may mediate their production of PGE_2. LPS, IL-1β, and IL-18 *per se* induced no PGE_2 release from Kc. In contrast, C alone and C + LPS, IL-1β, or IL-18 very quickly (<2.5 min) triggered similar PGE_2 increases. PGE_2 production in all cases was apparently undifferentially catalyzed by COX-1 and COX-2; i.e., the cells from WT, COX-1 and COX-2 null mice released PGE_2 in similar amounts and at similar rates (Kc express both COX-1 and –2 constitutively [126]). In contrast, PM responded later (10-30 min) than Kc and equally to C alone and C + LPS, IL-1β, or IL-18, most to LPS alone, and least to IL-1β and IL-18 alone; PGE_2 production was COX-2-dependent in all instances (PM express COX-1, but not COX-2 constitutively [126]).Thus, pyrogen-induced PGE_2 production is modulated differently in Kc and PM. In Kc, it occurs very rapidly, is mediated by both COX isoforms, and is entirely C-dependent. In PM, it develops significantly more slowly, is COX-2-mediated, and is C-independent. These results thus are in conformity with our *in vivo* findings, earlier.

7. SUMMARY AND PERSPECTIVE

Our principal findings can be summarized as follows:

1. C is a concentration-dependent, required mediator of the febrile response to LPS of guinea pigs and mice.
2. The absence of C5 and, specifically, the blockade of C5aR1 prevent the febrile responses of mice to iv and ip LPS. C5a thus appears to be the specific, critical mediator of LPS fever.
3. C5(a), however, does not have a role in the febrile responses to other, not Gram-negative-derived exogenous pyrogens.
4. Its site of action is peripheral, in the liver, rather than central, in the POA.
5. Kupffer cells (Kc), in particular, play a central role in the initiation of the febrile responses to both iv and ip LPS.
6. The avidity of Kc for LPS is influenced by the spleen and probably by neutrophils, but not by C(5a). It is related directly to fever height.
7. C3 activation by CVF injected ip, iv, or into the portal vein causes the immediate appearance of PGE2 in the inferior vena cava. LPS injected by the same routes evokes the same responses, correlatively with the onset of the febrile rise. Decomplementation prevents the rises of the LPS-induced rises of Tc and plasma PGE2. Pyrogenic cytokines appear after both C3 activation and LPS, but their courses are not correlated with the initial development of the febrile response.
8. C(5a), but not LPS, virtually immediately activates Kc to release PGE2. Cytokines appear later.

Taken together, these results are compatible with our working hypothesis that the febrile response to LPS may be initiated in the following sequential steps: 1) irrespective of its iv or ip route of entry, LPS is delivered to the liver; 2) Kc promptly bind it and C3 is activated locally; 3) focally localized C5a is consequently produced; 4) C5a then activates Kc to immediately release PGE_2 (pyrogenic cytokines are released later); 5) this PGE_2 (as yet presumptively) stimulates nearby vagal afferents that transmit the pyrogenic message to the POA.

To definitively ascertain this hypothesis, we must establish conclusively that: 1) C5a alone is the sole mediator that initiates this process, 2) Kc, and not other cell types in the liver, e.g., mast cells, are the specific target and the source of the presumptive secondary mediator, 3) this presumptive mediator is indeed PGE_2, and not another COX-2-derived prostanoid, e.g., PGD_2; and 4) it activates local vagal afferents. And we must be prepared for alternative results! These studies should help to elucidate the still largely unknown mechanisms that trigger the

febrile response to bacterial pathogens and also, by extension, the acute-phase reaction, as well as potentially point to ways in which the particularly untoward effects of bacterial infection might be mitigated therapeutically.

8. ACKNOWLEDGMENTS

The studies reported herein were supported by NIH grant NS #34857 to CMB. The authors thank Daniel Morse and Gregory Short for their excellent graphic arts support. CMB also wishes to express his sincere gratitude to his earlier co-workers, Elmir Sehic and Alberte Ungar, who contributed so much to the beginning of this project. He also gratefully acknowledges the support of V. Michael Holers, Susan A. Boackle, John D. Lambris, Leslie R. Ballou, and Sarita Goorha, who generously provided various gene-ablated mice and selective drugs as well as performed certain specialized assays.

8. REFERENCES

1. Blatteis C. M & E. Sehic: Fever: how may circulating pyrogens signal the brain? *News Physiol Sci* 12, 1-9 (1997)
2. Blatteis C. M, E. Sehic & S. Li: Pyrogen sensing and signaling: old views and new concepts. *Clin Infect Dis* 31, S168-S177 (2000)
3. Dunn A. J: Mechanisms by which cytokines signal the brain. *Int Rev Neurobiol* 52, 43-65 (2002)
4. Banks W. A, S. R. Plotkin & A. J. Kastin: Permeability of the blood-brain barrier to soluble cytokine receptors. *Neuroimmunomodulation* 2,161-165 (1995)
5. Banks W. A, A. J. Kastin, & R. D. Broadwell: Passage of cytokines across the blood-brain barrier. *Neuroimmunomodulation* 2, 241-248 (1995)
6. Blatteis C. M: Role of the OVLT in the febrile response to circulating pyrogens. *Prog Brain Res* 91, 409-412 (1992)
7. Blatteis C. M, S. L. Bealer, W. S. Hunter, J. Llanos-Q, R. A. Ahokas & T. A. Mashburn Jr: Suppression of fever after lesions of the anteroventral third ventricle in guinea pigs. *Brain Res Bull* 11, 519-526 (1983)
8. Romanovsky A. A, N. Sugimoto, C. T. Simons & W. S. Hunter: The organum vasculosum laminae terminalis (OVLT) in immune-to-brain febrigenic signaling: a reappraisal of lesion experiments. *Am J Physiol* 285, R420-R428 (2003)
9. Matsumura K, C. Cao, M. Ozaki, H. Morii, K. Nakadate & Y. Watanabe: Brain endothelial cells express cyclooxygenase-2 during lipopolysaccharide-induced fever: Light and electron microscopic immunocytochemical studies. *J Neurosci* 18, 6279-6287 (1998)
10. Li S, Y. Wang, K. Matsumura, L. R. Ballou, S. G. Morham & C. M. Blatteis: The febrile response to lipopolysaccharide is blocked in COX-2-/- but not in COX-1-/- mice. *Brain Res* 825, 86-94 (1999)
11. Elmquist J. K, C. D. Breder, J. E. Sherin, T. E. Scammell, W. F. Hickey, D. DeWitt & C. B. Saper: Intravenous lipopolysaccharide induces cyclooxygenase-2-like immunoreactivity in rat brain perivascular microglia and meningeal macrophages. *J Comp Neurol* 381, 119-129 (1997)
12. Ross G, T. Hubschle, U. Pehl, H. Braum, K. Voigt, R. Gerstberger & J. Roth: Fever induction by localized subcutaneous inflammation in guinea pigs: the role of cytokines and prostaglandins. *J Appl Physiol* 94, 1395-1402 (2003)
13. Sehic E, M. Székely, A. L. Ungar, A. Oladehin & C. M. Blatteis: Hypothalamic PGE2 during lipopolysaccharide-induced fever in guinea pigs. *Brain Res Bull* 39, 391-399 (1996)
14. Elmquist J. K, T. E. Scammell, C. D. Jacobson & C. B. Saper: Distribution of Fos-like immunoreactivity in the rat brain following intravenous lipopolysaccharide administration. *J Comp Neurol* 371, 85-103 (1996)
15. Jansky L, S. Vybiral, D. Pospisilova, J. Roth, J. Dorand & E. Zeisberger: Production of systemic and hypothalamic cytokines during the early phase of endotoxin fever. *Neuroendocrinology* 62, 55-61 (1995)
16. Givalois L, J. Dorand, M. Mekaouehe, M. D. Solier, A. F. Bristow, G. Ivart, P. Siand, J. Assenmacher, G. Barbanel: Temporal cascade of plasma level surges in ACTH, corticosterone, and cytokines in endotoxin-challenged rats. *Am J Physiol* 266, R164-R170 (1994)
17. Blatteis C. M, E. Sehic & S. Li: Complement and the pathogenesis of endotoxic fever. *Int J Biometerol* 43,176-183 (2000)
18. Yamagata K, K. Matsumura, W. Inoue, T. Shiraki, K. Suzuki, S. Yasuda, H. Sugiura, C. Cao, Y. Watanabe & S. Kobayashi: Coexpression of microsomal-type prostaglandin E synthase with cyclooxygenase-2 in brain endothelial cells of rats during endotoxin-induced fever. *J Neurosci* 21, 2669-2677 (2001)
19. Sehic E & C. M. Blatteis: Blockade of lipopolysaccharide-induced fever by subdiaphragmatic vagotomy in guinea pigs. *Brain Res* 726, 160-166 (1996)
20. Niijima A: The afferent discharges from sensors for interleukin-1b in the hepatoportal system in the anesthetized rat. *J Autonom Nerv Syst* 61, 287-291 (1996)
21. Wan W. A, L. Wetmore, C. M. Sorensen, A. H. Greenberg & D. M. Vance: Neural and biochemical mediators of endotoxin and stress-induced c-fos expression in the rat brain. *Brain Res Bull* 34, 7-141(994)
22. Watkins L. R, L. E. Goehler, J. K. Reston, N. Tartaglia, L. Gilbert, D. Martin & S. F. Maier: Blockade of interleukin-1-induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. *Neurosci Lett* 183, 27-31 (1995)
23. Gaykema R. P. A, I. Dijkstra & F. J. H. Tilders: Subdiaphragmatic vagotomy suppresses endotoxin-induced activation of hypothalamic corticotropin-releasing hormone neurons and ACTH secretion. *Endocrinology* 136, 4717-4720 (1995)
24. Ek M, M, Kurosowa, T. Lundeberg & A. Ericson: Activation of vagal afferents after intravenous injection of interleukin-1b: role of endogenous prostaglandins. *J Neurosci* 18, 9471-9479 (1998)
25. Simons C. T, V. A. Kulchitsky, N. Sugimoto, L. D. Homer, M. Szekely & A. A. Romanovsky: Signaling the brain in systemic inflammation: which vagal branch is involved in fever genesis? *Am J Physiol* 275, R63-R68 (1998)
26. Goehler L. E, J. K. Relton, D. Dripps, R. Kiechle, N. Tartaglia, S. F. Maier& L. R. Watkins: Vagal paraganglia

bind biotinylated interleukin-1 receptor antagonist: a possible mechanism for immune-to-brain communication. *Brain Res Bull* 43, 357-364 (1997)
27. Szekely M, M. Balasko, V. A. Kulchitsky, C. T. Simons, A. I. Ivanov & A. A. Romanovsky: Multiple neural mechanisms of fever. *Auton Neurosci* 85, 78-82 (2000)
28. Blatteis C. M, E. Sehic & S. Li: Afferent pathways of pyrogen signaling. *Ann NY Acad Sci* 856, 95-107 (1998)
29. Gallay P, C. V. Jongencel, C. Barras, M. Burnier, J-D. Baumgarter, M. P. Glauser & D. Heumann: Short-term exposure to lipopolysaccharide is sufficient to activate human monocytes. *J Immunol* 150, 5086-5093 (1993)
30. Callery M. P, M. J. Mangino & M. W. Flye: A biological basis for limited Kupffer cell reactivity to portal-derived endotoxin. *Surgery* 110, 221-230 (1991)
31. Fox E. S, P. Thomas & S. A. Broitman: Comparative studies of endotoxin uptake by isolated rat Kupffer and peritoneal cells. *Infect Immun* 55, 2962-2966 (1987)
32. Blatteis C. M, N. Quan, L. Xin & A. L. Ungar: Neuromodulation of acute-phase responses to interleukin-6 in guinea pigs. *Brain Res Bull* 25, 895-901 (1990)
33. Coceani F & E. S. Akarsu: Prostaglandin E2 in the pathogenesis of fever: an update. *Ann NY Acad Sci* 856, 76-82 (1998)
34. Kramer R. M: Structures and function of Ca2+-sensitive cytosolic phospholipase A2. In: Signal-Activated Phospholipases. Eds: Landes R. G, Liscovitch, Austin (1994)
35. Watson R. W. G, H. P. Redmond & D. Bouchier-Hayes: Role of endotoxin in mononuclear phagocyte-mediated inflammatory responses. *J Leukocyte Biol* 56, 95-103 (1994)
36. Rotondo D, H. T. Abul, A. S. Milton & J. Davidson: Pyrogenic immunomodulators increase the level of prostaglandin E2 in the blood simultaneously with the onset of fever. *Eur J Pharmacol* 154, 145-152 (1988)
37. Lacroix S & S. Rivest: Effect of acute systemic inflammatory response and cytokines on the transcription of the genes encoding cyclooxygenase enzymes (COX-1 and COX-2) in the rat brain. *J Neurochem* 70, 452-466 (1998)
38. Masferrer J. L, K. Seibert, B. Zweifel & P. Needleman: Endogenous glucocorticoids regulate an inducible cyclooygenase enzyme. *Proc Natl Acad Sci USA* 89, 3917-3921 (1992)
39. Smith W. L & D. L. DeWitt: Biochemistry of prostaglandin endoperoxide H-synthase-1 and synthase-2 and their differential susceptibility to nonsteroidal antiinflammatory drugs. *Semin Nephrol* 15,179-194 (1995)
40. O'Bannion M. K: Cyclooxygenase-2: molecular biology pharmacology, and neurobiology. *Crit Rev Neurobiol* 13, 45-82 (1999)
41. Chandrasekharan N. V, H. Dai, K. L. Roos, N. K. Evanson, J. Tomsik, T. S. Elton, & D. L. Simmons: COX-3, a cyclooxygenase-1 variant inhibited by acetominophen and other analgesic/antipyretic drugs: cloning, structure, and expression. *PNAS* 99, 13926-13931 (2002)
42. Blatteis C. M, E Sehic& Li S: Kupffer cells and complement mediate the febrile response of guinea pigs to endotoxin. In: Thermal Physiology 1997. Eds: Nielsen J. B, Nielsen R, The August-Krogh Inst Press, Copenhagen, DK (1997)
43. Vukajlovich S. W: Interaction of LPS with serum complement. In: Bacerial Endotoxic Lipopolysaccharides. Eds: Ryan J. L, Morrison D. C, CRC: Boca Raton, FL, vol. II, 213-235 (1992)
44. Hinglas N, M. D. Kazatchkine, C. Mandel, M-D. Appay & J. Bariety: Human Kupffer cells express CR1, CR3, and CR4 complement receptor antigens. An immunohistochemical study. *Lab Invest* 61, 509-514 (1989)
45. Püschel G. P, U. Hespeling, M. Oppermann & P. Dieter: Increase in prostanoid formation in rat liver macrophages (Kupffer cells) by human anaphylatoxin C3a. *Hepatology* 18,1516-1521 (1993)
46. Hänsch G. M, M. Seitz, G. Martinotti, M. Betz, E. W. Rautenberg & D. Gemsa: Macrophages release arachidonic acid, prostaglandin E2, and thromboxane in response to late complement components. *J Immunol* 133, 2145-2150 (1984)
47. Fink M. P, H. R. Rothschild, Y. F. Deniz, S. M. Cohn: Complement depletion by naja haje cobra venom factor limits prostaglandin release and improves visceral perfusion in porcine endotoxic shock. *J Trauma* 29,1076-1085 (1989)
48. Schieferdecker H. L, G. Schlaf, K. Jungermann & O. Gotze: Functions of anaphylatoxin C5a in rat liver: direct and indirect actions on nonparenchymal and parenchymal cells. *Int Immunopharmacol* 1, 469-481 (2001)
49. Schlaf G, M. Schmitz, E. Rothermel, K. Jungermann, H. Schieferdecker & O. Gotze: Expression and induction of anaphylatoxin C5a receptors in the rat liver. *Histol Histopathol* 18, 299-308 (2003)
50. Ushikubi F, E. Segi, Y. Sugimoto, T. Murata, T. Matsuoka, T. Kobayashi, H. Hizaki, K. Tuboi, M. Katsuyama, A. Ishikawa, T. Tanaka, N. Yoshida & S. Narumiya: Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. *Nature* 395, 281-284 (1998)
51. Oka T, K. Oka & C. B. Saper: Constrasting effects of E type prostaglandin (EP) receptor agonists on core body temperature in rats. *Brain Res* 968, 256-262 (2003)
52. Oka T & T. Hori: EP1-receptor mediation of prostaglandin E2-induced hyperthermia in rats. *Am J Physiol* 267, R289-R294 (1994)
53. Oka K, T. Oka & T. Hori: PGE2 receptor subtype EP1 antagonist may inhibit central interleukin-1beta-induced fever in rats. *Am J Physiol* 275, R1762-R1765 (1998)
54. Oka T, K. Oka, T. E. Scammell, C. Lee, J. F. Kelly, F. Nantel, J. K. Elmquist & C. B. Saper: Relationship of EP(1-4) prostaglandin receptors with rat hypothalamic cell groups involved in lipopolysaccharide fever responses. *J Comp Neurol* 428, 20-32 (2000)
55. van den Berg C. W, P. C. Aerts & H. van Dijk: In vivo anti-complementary activities of the cobra venom factors from Naja naja and Naja haje. *J Immunol Methods* 136, 287-294 (1991)
56. Vogel C. W, R. Bredehorst, D. C. Fritzinger, T. Grunwald, P. Ziegelmuller & M. A. Koek: Structure and function of cobra venom factor, the complement-activating protein in cobra venom. *Adv Exp Med* 391, 97-114 (1996)

57. Li S, E. Sehic, Y. Wang, A. L. Ungar & C. M. Blatteis: Relation between complement and the febrile response of guinea pigs to systemic endotoxin. *Am J Physiol* 277, R1635-R1645 (1999)
58. Sehic E, S. Li, A. L. Ungar & C. M. Blatteis: Complement reduction impairs the febrile response of guinea pigs to endotoxin. *Am J Physiol* 274, R1594-R1603 (1998)
59. Li S, E. Sehic, A. L. Ungar & C. M. Blatteis: Complement does not mediate the febrile responses of guinea pigs to muramyl dipeptide and polyriboinosinic-polyribocytidylic acid. *J Thermal Biol* 25, 51-58 (2000)
60. Sehic E, W. S. Hunter, A. L. Ungar & C. M. Blatteis: Blockade of Kupffer cells prevents the febrile and preoptic prostaglandin E2 responses to intravenous lipopolysaccharide in guinea pigs. *Ann NY Acad Sci* 813, 448-452 (1997)
61. Cochrane C. G, H. J. Muller-Eberhard & B. S. Aikin: Depletion of plasma complement in vivo by a protein from cobra venom: its effect on various immunological reactions. *J Immunol* 105, 55-69 (1970)
62. Goldbach J, J. Roth & E. Zeisberger: Fever suppression by subdiaphragmatic vagotomy in guinea pigs depends on the route of pyrogen administration. *Am J Physiol* 272, R675-R681 (1997)
63. Fenton M. J & D. T. Golenbock: LPS-binding proteins and receptors. *J Leukocyte Biol* 64, 25-32 (1998)
64. Schumann R. R, E. T. Rietschel & H. Loppnew: The role of CD14 and lipopolysaccharide-binding protein (LBP) in the activation of different cell types by endotoxin. *Med Microbiol Immunol* 183, 279-297 (1994)
65. Ingalls R. R, M. A. Arnaout, R. L. Delude, S. Flaherty, R. Savedra Jr & D. T. Golenbock: The CD11/CD18 integrins: characterization of three novel LPS signaling receptors. *Prog Clin Biol Res* 397, 107-117 (1998)
66. Ingalls R. R & D. T. Golenbock: CD11c/CD18, a transmembrane signaling receptor for lipopolysaccharide. *J Exp Med* 181, 1473-1479 (1995)
67. Haziot A, E. Ferrero, F. Kontgen, N. Hijiya, S. Yamamoto, J. Silver, C. L. Stewart & S. M. Goyert: Resistance to endotoxin shock and reduced dissemination of Gram-negative bacteria in CD14-deficient mice. *Immunity* 4, 407-414 (1996)
68. Ryozo M, Y. Aramaki, H. Arima, Y. Adachi, N. Ohno, T. Yadomae & S. Tsuchiya: Contribution of CR3 to nitric oxide production from macrophages stimulated with high-doses of LPS. *Biochem Biophys Res Commun* 244,115-119 (1998)
69. Wright S. D, S. M. Levin, M. T. C. Jong, E. Chad & L. G. Kabbash: CR3 (CD11c/CD18) expresses one binding site for Arg-Gly-Asp-containing peptides and a second site for bacterial lipopolysaccharide. *J Exp Med* 169, 175-183 (1989)
70. Bilsland C. A, M. S. Diamond & T. A. Springer: The leukocyte integrin p150, 95 (CD11c/CD18) as a receptor for iC3b. Activation by a heterologous beta subunit and localization of a ligand recognition site to the I domain. *J Immunol* 152, 4582-4589 (1994)
71. Couturier C, N. Haeffner-Cavaillon, L. Weiss, E. Fischer & M. D. Kazatchkine: Induction of cell-associated interleukin-1 through stimulation of the adhesion-promotion proteins LFA-1 (CD11a/CD18) and CR3 (CD11b/CD18) of human monocytes. *Eur J Immunol* 20, 999-1005 (1990)
72. Wright S. D: Receptors for complement and the biology of phagocytosis. In: Inflammation: Basic Principles and Clinical Correlates. Eds: Gallin J. I, Goldstein I. M, Snyderman R, Raven, New York, 2nd ed, 477-495 (1992)
73. Roth J, S. J. Hopkins, M. E. Hoadley, A. Tripp, T. Aslan, B. Storr, G. Luheshi & E. Zeisberger: Fever and production of cytokines in response to repeated injections of muramyl dipeptide in guinea pigs. *Eur J Physiol* 434, 525-533 (1997)
74. Kimura M, L. A. Toth, H. Agoshini, A. B. Cady, J. A. Majde & J. M. Krueger: Comparison of acute-phase response mediated in rabbits by lipopolysaccharide and double-stranded RNA. *Am J Physiol* 267, R1596-R1605 (1994)
75. Leu R. W, J. A. Rummage, M. B. Rahimi & M. J. Herriott: Relationship between murine macrophage Fc receptor-mediated phagocytic function and competency for activation for non-specific tumor cytotoxicity. *Immunobiology* 171, 220-233 (1986)
76. Williams B. B, R. T. Paul & A. M, Lerner: Pharmacokinectics of interferon in blood, cerebrospinal fluid, and brain after administration of modified polyriboinosinic-polyribocytidylic acid and amphotericin B. *J Infect Dis* 146, 819-825 (1982)
77. Krueger J. M, L. Johannsen, L. A. Toth, M. Opp & L. Kapas: CNS mechanisms for sleep responses in microbrial challenge. In: Peripheral Signaling of the Brain: Neural-immune and Cognitive Functions, Eds: Frederickson R. C. A, McGaugh J. L, Felten D. L, Hogrefe and Huber, Toronto, 179-192 (1991)
78. Gegout P, P. Gillet, D. Chevrier, C. Guingamp, B. Terlain & P. Netter: Characterization of zymosan-induced arthritis in the rats: effects on joint inflammation and cartilage metabolism. *Life Sci* 55, PL 321-326 (1994)
79. Li S, J. Llanos-Q & C. M. Blatteis: Thermal response to zymosan: the differential role of complement. *Neuroimmunomodulation* 10, 122-128 (2002-03)
80. Li, S, V. M. Holers, S. A. Boackle & C. M. Blatteis: Mediation of mouse endotoxic fever by complement. *Infect Immun* 70, 2519-2525 (2002)
81. Li, S & C. M. Blatteis: Complement (C) 3 is involved in the febrile response of mice to intraperitoneal (ip), but not intravenous (iv) lipopolysacchride (LPS). *FASEB* J 16, A873 (2002)
82. Li, S, J. D. Lambirs & C.M. Blatteis: Complement and lipopolysaccharide-induced fever: C5a is essential. *FASEB* J 17, C108 (2003)
83. Li, S, S. A. Boackle, V. M. Holers, J. D. Lambris & C. M. Blatteis: Complement 5a is integral to the febrile response of mice to lipopolysaccharide. *Am J Physiol* (submmited, 2003)
84. Nadeau S & S. Rivest: The complement system is an integrated part of the natural innate immune response in the brain. *FASEB* J 15, 1410-1412 (2001)
85. Li, S & C. M. Blatteis: Complement (C) 5 is necessary for the febrile response of mice to intravenously (iv), but not intracerebroventricularly (icv), injected lipopolysaccharide (LPS). *J Endotoxin Res* 8, 185 (2002)
86. Kitamura K, M. Matsumoto & K. Nagaki: C3-independent immune hemolysis EAC14oxy2 cells by C5-

C9 without participation of C3. *Immunology* 53, 575-582 (1984)
87. Kitamura K, M. Tsuboi & K. Nagaki: The activation of C5 in the fluid phase and in the absence of C3 through the classical pathway of the complement system. *Immunology* 88, 459-465 (1986)
88. Masaki T, M. Matsumoto, R. Yasuda, R. P. Levine, H. Kitamura & T. Sepa: A covalent dimer of complement C4b serves as a subunit of a novel C5 convertase that involves no C3 derivatives. *J Immunol* 147, 927-932 (1991)
89. Li Z & C. M. Blatteis: Fever onset is linked to the appearance of lipopolysaccharide in the liver. *J Endotoxin Res* (in press)
90. Feleder C, Z. Li, V. Perlik, A. Evans & C. M. Blatteis: The spleen modulates the febrile response of guinea pigs to LPS. *Am J Physiol* 284, R1466-R1476 (2003)
91. Colten H. R, Y. M. Ooi & P. J. Edelson: Synthesis and secretion of complement proteins by macrophages. *Ann NY Acad Sci* 332, 482-490 (1979)
92. Hetland G, E. Johnson & U. Aasebo: Human alveolar macrophages synthesize the functional alternative pathway of complement and active C5 and C9 in vitro. *Scand J Immunol* 24, 603-608 (1986)
93. Hetland G, E. Johnson, P. Royset & T. Eskeland: Human alveolar macrophages and monocytes generate the functional classical pathway of complement in vitro. *Acta Path Microbiol Immunol Sacnd* 95, 117-122 (1987)
94. Ooi Y. M, D. E. Harris, P. J. Edelson & H. R. Colten: Post-translational control of complement (C5) production by resident and stimulated mouse macrophages. *J Immunol* 124, 2077-2081 (1980)
95. Whaley K: Biosynthesis of the complement components and the regulatory proteins of the alternative complement pathways by human peripheral blood monocytes. *J Exp Med* 151, 501-516 (1980)
96. Huber-Lang M, E. M. Younkin, J. V. Sarma, N. Riedermann, S. R. McGuire, K. T. Lu, R. Kunkel, J. G. Younger, F. S. Zetoune & P. A. Ward: Generation of C5a by phagocytic cells. *Am J Pathol* 161, 1849-1859 (2002)
97. Haziot A, B. Z. Tsuberi & S. M. Goyert: Neutrophil CD14: biochemical properties and role in the secretion of tumor necrosis factor-alpha in response to lipopolysaccharide. *J Immunol* 150, 5556-5565 (1993)
98. Wiggins R. C, P. C. Giclas & P. M. Henson: Chemotactic activity generated from the fifth component of complement by plasma kallikrein of the rabbit. *J Exp Med* 153, 1391-1404 (1981)
99. Wingrove J. A, R. G. DiScipio, Z. Chen, J. Potempa, J. Travis & T. E. Hugli: Activation of complement components C3 and C5 by a cysteine proteinase (gingipain-1) from porphyromonas (bacteroides) gingivalis. *J Biol Chem* 267, 18902-18907 (1992)
100. Schalf G, H. L. Schieferdecker, E. Rothermel, K. Jungermann & O. Gotze: Differential expression of the C5a receptor on the main cell types of rat liver as demonstrated with a novel monoclonal antibody and by C5a anaphylatoxin-induced Ca2+ release. *Lab Invest* 79, 1287-1297 (1999)
101. Schieferdecker H. L, E. Rothermel, A. Timmermann, O. Gotze & K. Jungermann: Anaphylatoxin C5a receptor mRNA is strongly expressed in Kupffer and stellate cells and weakly in sinusoidal endothelial cells but not in hepatocytes of normal rat liver. *FEBS Lett* 406, 305-308 (1997)
102. Mathison J. C & R. J. Ulevitch: The clearance, tissue distribution, and cellular localization of intravenously injected lipopolysaccharide in rabbits. *J Immunol* 123, 2133-2143 (1979)
103. Mathison J. C, R. J. Ulevitch, J. R. Fletcher & C. G. Cochrane: The distribution of lipopolysaccharide in normocomplementemic and C3-depleted rabbits and rhesus monkeys. *Am J Pathol* 101, 245-262 (1980)
104. Fox E. S, P. Thomas & S. A. Broitman: Hepatic mechanisms for clearance and detoxification of bacterial endotoxins. *J Nutr Biochem* 1, 620-628 (1990)
105. Armbrust T & G. Ramadori: Functional characterization of two different Kupffer cell populations of normal rat liver. *J Hepatol* 25, 518-528 (1996)
106. Bioulac-Sage P, J. Kuiper, T. J. C. Van Berkel & C. Balabaud: Lymphocyte and macrophage populations in the liver. *Hepatogastroenterology* 43, 4-14 (1996)
107. Hardonk M. J, F. W. J. Dijkhuis, C. E. Hulstaert & J. Koudstaal: Heterogeneity of rat liver and spleen macrophages in gadolinium chloride-induced elimination and repopulation. *J Leukocyte Biol* 52, 296-302 (1992)
108. Lenczowski M. J. P, A. M. Van Dam, S. Poole, J. W. Larrick & F. J. H. Tilders: Role of circulating endotoxin and interleukin-6 in the ACTH and corticosterone response to intraperitoneal LPS. *Am J Physiol* 273, R1870-R1877 (1997)
109. Hansen M. K, K. T. Nguyen, M. Fleshner, L. E. Goehler, R. P. Gaykema, S. F. Maier & L. R. Watkins: Effects of vagotomy on serum endotoxin, cytokines and corticosterone after intraperitoneal lipopolysaccharide. *Am J Physiol* 178, R331-R336 (2000)
110. Romanovsky A. A, A. I. Ivano, M. J. P. Lenczowski, V. A. Kulchitsky, A-M. vanDam, S. Poole, L. D. Homer & F. J. Tilders: Lipopolysaccharide transport from the peritoneal cavity to the blood: is it controlled by the vagus nerve? *Auton Neurosci* 85, 133-140 (2000)
111. Daniele R, H. Singh, H. E. Appert, F. N. Pairent & J. M. Howard: Lymphatic absorption of intraperitoneal endotoxin in the dog. *Surgery* 67, 484-487 (1970)
112. Olofsson P, G. Nylander & P. Olsson: Endotoxin: routes of transport in experimental peritonitis. *Am J Surg* 151, 443-447 (1986)
113. Dinarello C. A, P. Bodel & E. Atkins: The role of the liver in the production of fever and in pyrogenic tolerance. *Trans Assoc Am Physicians* 81, 334-344 (1968)
114. Derijk R. H, P. J. L. M. Strijbos, N. V. Rooijen, N. J. Rothwell & F. Berkenbosch: Fever and thermogenesis in response to bacterial endotoxin involve macrophage-dependent mechanisms in rats. *Am J Physiol* 265, R1179-R1183 (1993)
115. Székely M, E. Kalman & F. Liszt: Liver function and fever pathogenesis. *Pathophysiology* 5, 273 (1998)
116. Ge Y, R. M. Ezzell, B. D. Clark, P. M. Loiselle, S. F. Amato & HS. Warren: Relationship of tissue and cellular interleukin-1 and lipopolysaccharide after endotoxemia and bacteremia. *J Infect Dis* 176, 1313-1321 (1997)
117. Billiar T. R, M. A. West, B. J. Hyland & R. L. Simmons: Splenectomy alters Kupffer cell response to endotoxin. *Arch Surg* 123, 327-332 (1988)
118. Feleder C, Z. Li, V. Perlik, A. Evans & C. M. Blatteis: The spleen modulates the febrile response of guinea pigs to LPS. *Am J Physiol* 284, R1466-R1476 (2003)

119. Meekes I, F. van der Staak & C. van Oostrom: Results of splenectomy performed on a group of 91 children. *Eur J Pediatr Surg* 5, 19-22 (1995)
120. Matsukawa A & M. Yoshinago: Sequential generation of cytokines during the initial phase of inflammation, with reference to neutrophils. *Inflam Res* 47, S137-S144 (1998)
121. Malcolm K. C, P. G. Arndt, E. J. Manos, D. A. Jones & G. S. Worthen: Microarray analysis of lipopolysaccharide-treated human neutrophils. *Am J Physiol* 284, L663-L670 (2003)
122. Brown A. E: Management in the febrile, neutropenic patient with cancer: therapeutic considerations. *J Pediatr* 106, 1035-1042 (1985)
123. Zanetti G, D. Heumann, J. Gérain, J. Kohler, P. Abbet, C. Barras, R. Lucas, M. P. Glauser & J. D. Baumgartner: Cytokine production after intravenous or peritoneal Gram-negative bacterial challenge in mice: comparative protective efficacy of antibodies to tumor necrosis factor-a and to lipopolysaccharide. *J Immunol* 148, 1890-1897 (1992)
124. Feleder C, V. Perlik & C. M. Blatteis: Intrapreoptic norepinephrine induces body temperature rises via COX-2-dependent PGE2 in guinea pigs. *Am J Physiol* (submitted, 2003)
125. Perlik V, Z. Li, S. Goorha, L. R. Ballou & Blatteis C. M: Lipopolysaccharide (LPS)-activated complement (C) rather than LPS per se initiate the febrile response. *J Leukocyte Biol* (in press)
126. Ahmad N, L. C. Chen, M. A. Gordon, J. D. Laskin & D. L. Laskin: Regulation pf cyclooxygenase-2 by nitric oxide in activated hepatic macrophages during acute endotoxemia. *J Leukocyte Biol* 71, 1005-1011 (2002)

Key Words: Fever, LPS, Kupffer cells, Macrophages, PGE_2, Cytokines, Vagus, Liver, Spleen, Neutrophilia, Review

Send correspondence to: Clark M. Blatteis, Department of Physiology, College of Medicine, University of Tennessee Health Science Center, 894 Union Avenue, Memphis, TN 38163, USA, Tel: 901-448-5845, Fax: 901-448-1673. E-mail: blatteis@physio1.utmem.edu