PNAS RESEARCH ARTICLE | IMMUNOLOGY AND INFLAMMATION

sEH-derived metabolites of linoleic acid drive pathologic inflammation while impairing key innate immune cell function in burn injury

Christian B. Bergmann[a,1], Cindy B. McReynolds[b,1], Debin Wan[b], Nalin Singh[b], Holly Goetzman[a], Charles C. Caldwell[a], Dorothy M. Supp[c,d], and Bruce D. Hammock[b,e,2]

Contributed by Bruce D. Hammock; received November 15, 2021; accepted February 16, 2022; reviewed by Bernie Hennig and Mehran Moghaddam

Fatty acid composition in the Western diet has shifted from saturated to polyunsaturated fatty acids (PUFAs), and specifically to linoleic acid (LA, 18:2), which has gradually increased in the diet over the past 50 y to become the most abundant dietary fatty acid in human adipose tissue. PUFA-derived oxylipins regulate a variety of biological functions. The cytochrome P450 (CYP450)–formed epoxy fatty acid metabolites of LA (EpOMEs) are hydrolyzed by the soluble epoxide hydrolase enzyme (sEH) to dihydroxyoctadecenoic acids (DiHOMEs). DiHOMEs are considered cardioprotective at low concentrations but at higher levels have been implicated as vascular permeability and cytotoxic agents and are associated with acute respiratory distress syndrome in severe COVID-19 patients. High EpOME levels have also correlated with sepsis-related fatalities; however, those studies failed to monitor DiHOME levels. Considering the overlap of burn pathophysiology with these pathologies, the role of DiHOMEs in the immune response to burn injury was investigated. 12,13-DiHOME was found to facilitate the maturation and activation of stimulated neutrophils, while impeding monocyte and macrophage functionality and cytokine generation. In addition, DiHOME serum concentrations were significantly elevated in burn-injured mice and these increases were ablated by administration of 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU), a sEH inhibitor. TPPU also reduced necrosis of innate and adaptive immune cells in burned mice, in a dose-dependent manner. The findings suggest DiHOMEs are a key driver of immune cell dysfunction in severe burn injury through hyperinflammatory neutrophilic and impaired monocytic actions, and inhibition of sEH might be a promising therapeutic strategy to mitigate deleterious outcomes in burn patients.

soluble epoxide hydrolase | burn injury | TPPU | DiHOME

Significance

Oxylipins alter immune cell function and potentially drive pathophysiology in burn and sepsis patients. Past and recent data reveal a correlation between increased systemic EpOME levels and reduced survival in human burn trauma and sepsis. This work extends these studies and provides evidence that the downstream sEH-derived metabolites, DiHOMEs, are driving worsening outcomes by altering the immune response. Inhibiting DiHOME metabolite formation with the sEH inhibitor, 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU), restored immune function by increasing immune cell survival and function. These data support the hypothesis that sEH-derived linoleic acid diols are responsible for increased mortality in burn and sepsis patients and also provide a rationale for testing the therapeutic blockage of DiHOME generation in burn and sepsis patients to improve their outcomes.

Interest in dietary fatty acid composition began in the 1950s when the American Heart Association (AHA) formed the Nutrition Committee to investigate the rising increase in heart disease. Based on its assessment, the AHA recommended reducing the intake of saturated fats and increasing intake of apparently healthier poly- and monounsaturated fatty acids. This factor, combined with agricultural practices driven toward corn production and a dietary preference toward processed foods, shifted fatty acid composition in the modern Western diet from saturated and monounsaturated to increasing concentrations of polyunsaturated fatty acids (PUFAs), specifically omega-6 linoleic acid (LA, 18:2), resulting in a corresponding increase in LA in human adipose composition since the 1950s (1). The metabolism of PUFAs results in numerous regulatory lipid mediators that influence inflammation and metabolism, and many observational studies have found associations between concentrations of parent PUFAs, their metabolites, and numerous diseases (2, 3). For example, higher dietary intake of omega-3 fatty acids found predominantly in cold-water fish is associated with better health outcomes and antiinflammatory properties (4), while the omega-6 fatty acids and their metabolites are associated with more complex phenotypes. Omega-3 and -6 PUFAs are metabolized primarily by the cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP) oxidation enzymes, and secondary metabolism pathways generate further regulatory metabolites (for review, see ref. 5). The metabolism of LA specifically results in multiple biologically relevant compounds associated with numerous diseases (6). Specifically, the CYP metabolism pathway of LA has recently been of considerable interest with the discovery that LA-derived metabolites prevent heart disease partially through regulation of brown fat adipogenesis and thermogenesis (7).

The CYP metabolites of LA, epoxyoctadecenoic acids (EpOMES), were originally termed leukotoxins because these bioactive compounds were initially identified in

Reviewers: B.H., University of Kentucky; and M.M., OROX Biosciences.

Competing interest statement: C.B.M. and B.D.H. work with EicOsis on the development of epoxide hydrolase inhibitors for clinical use.

[1]C.B.B. and C.B.M. contributed equally to this work.

[2]To whom correspondence may be addressed. Email: bdhammock@ucdavis.edu.

This article contains supporting information online at http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2120691119/-/DCSupplemental.

Published March 21, 2022.

bacterial infections and found to cause an increase in leukocyte cytotoxicity. However, those original experiments did not investigate the downstream metabolites of EpOMEs. EpOMEs are metabolized by the soluble epoxide hydrolase (sEH) into corresponding vicinal diols, dihydroxyoctadecenoic acids (DiHOMEs) or leukotoxin diols. In 1997, Moghaddam et al. demonstrated that the cytotoxicity of the "leukotoxins" was in fact a result of sEH metabolism into the DiHOMEs, and if this metabolism was prevented through inhibition of the sEH, EpOMEs did not demonstrate cytotoxicity in alveolar cells (8). This was confirmed in animal models when intravenous injections of leukotoxin in healthy mice caused acute respiratory distress syndrome (ARDS) and ultimately death; however, inhibition of the sEH, either chemically or through genetic mutations, prevented this toxicity. Administration of DiHOMEs directly resulted in the same ARDS phenotype, at lower concentrations and independently of sEH inhibition (9). ARDS is a severe respiratory syndrome associated with high mortality from a wide range of primary insults, including sepsis, COVID-19 pneumonia, and burn injuries. Increased DiHOME concentrations have been observed in humans with severe COVID-19 infection compared to healthy controls (10), and lipidomic analyses in sepsis and burn patients identified an increase in EpOME concentrations correlating to higher incidence of mortality; however, DiHOMEs were not monitored in these patients (11).

Few research studies have focused on the role of diols in disease; however, recently Bergmann et al. discovered that sEH-derived diols from arachidonic acid metabolism impaired neutrophil function (12). The research described here characterized the oxylipin profile in the mouse burn model compared to sham-treated animals to determine whether mouse models mimic the increased LA-derived oxylipin metabolites observed in human burn patients, as well as characterized the immune-altering response of DiHOMEs to determine whether they could contribute to worse outcomes in burn patients. We selected the mouse burn model because of previously published papers describing the correlation between leukotoxin (EpOME) concentrations and worsening outcomes in burn patients (11). Burn injury results in a dysfunctional pattern of hyperinflammation (13, 14), thus our hypothesis was to use the mouse model of burn injury to test whether leukotoxin diols (DiHOMEs) were responsible for the dysregulated immune response in burn injury.

Results

PUFA Metabolites from Both Omega-3 and -6 Fatty Acids Are Increased in Scalded Mice, and LA-Derived Diols Are Reduced with Inhibition of sEH. Lipidomic analysis was conducted in serum of scalded or sham-treated mice that were treated prophylactically with three intraperitoneal (i.p.) injections of 10 mg/kg 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU) (sEH inhibitor) or vehicle (controls). Ninety-seven oxylipins were monitored quantitively for effects of scald injury and sEH-inhibitor treatment (Fig. 1*A*). The only COX-generated metabolite that significantly increased after scald treatment was prostaglandin B2. This compound was not altered by sEH-inhibitor administration. The remaining significantly altered regulatory lipids were generated from CYP- and sEH-mediated metabolism of PUFA. The diols derived from the omega-3 fatty acid, docosahexaenoic acid (DHA), increased after scald treatment (10,11-, 13,14-, and 19,20-dihydroxy-docosapentaenoic acid (DiHDPE)), and the DHA epoxide 19,20-epoxy-docosapentaenoic acid (EpDPE), also significantly

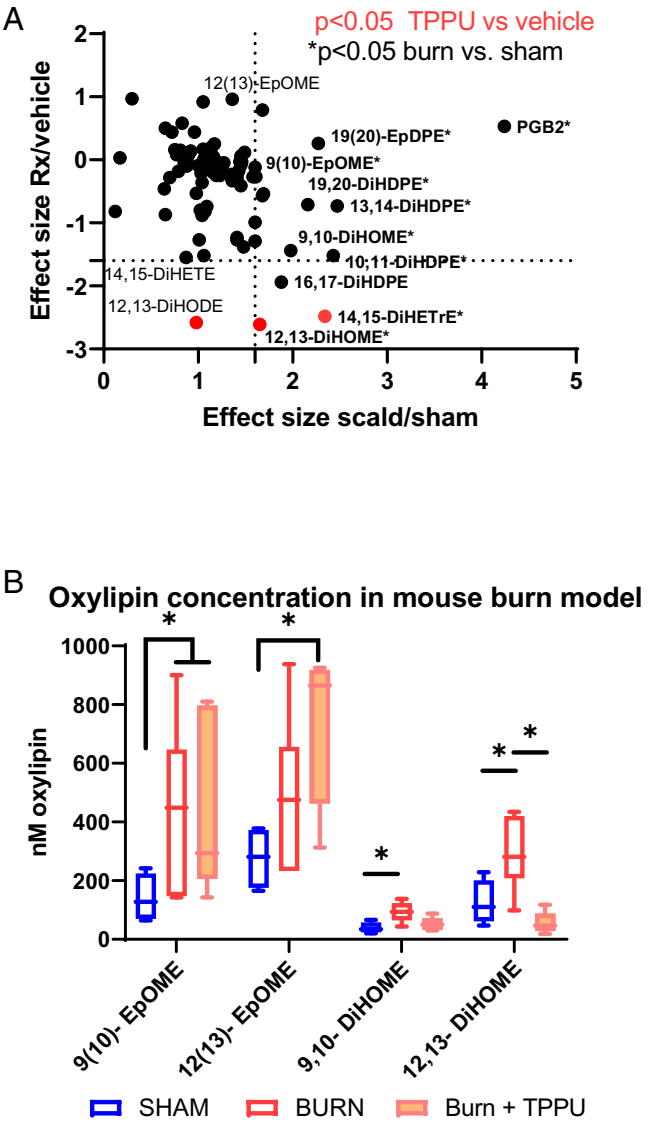

Fig. 1. Increased DiHOME concentrations after burn injury are reversed by sEH inhibitor, TPPU. Male C57BL/6N mice underwent scald injury resulting in a third-degree burn of 28% TBSA. The intervention group (n = 4) received intraperitoneal administration of TPPU at 48 h, 24 h, and directly before burn injury, while the control group (n = 4) received the vehicle PEG400. Twenty-four hours after the scald treatment, blood was harvested from all groups and centrifuged to gain serum, which was analyzed via mass spectroscopy to determine oxylipin serum levels. (*A*) The data were normalized for sham (x axis) and vehicle treatment (y axis), and the effect size for burn injury vs. sham was displayed on the x axis, and the effect size for TPPU treatment was shown on the y axis. An effect size of −1.6 (y axis) and 1.6 (x axis) was considered biologically relevant and marked using a dotted line. (*B*) Quantitative values of EpOMEs and DiHOMEs were evaluated. Data are presented as box plots with minimum to maximum values. *P < 0.05. Individual oxylipin values are reported in *SI Appendix*.

increased, indicating that the linoleate epoxides outcompete turnover by the sEH enzyme. The only DHA-derived metabolite that changed in response to burn and sEH treatment was 10,11-DiHDPE, and the only arachidonic acid metabolite in the CYP metabolism pathway to respond to burn was 14,15-dihydroxyicosa-tetraenoic acid (DiHETrE). This compound also decreased in response to sEH inhibition, consistent with previously published data (12). Both LA-derived diols (9,10-DiHOME and 12,13-DiHOME) increased in response to scald, but only the 12,13-DiHOME significantly decreased after sEH inhibition (Fig. 1*A*).The concentrations of 9,10 DiHOME also decreased, although not significantly. Considering the previous

implications of LA metabolites in ARDS and sepsis, effects on immune function were further analyzed in cells treated exogenously with biologically active EpOME and DiHOME.

TPPU Decreases Necrosis in Innate and Adaptive Immune Cells in Scalded Mice. Scalded and sham-burned mice were treated prophylactically three times with vehicle or 10 mg/kg TPPU i.p. to monitor the effects on innate and adaptive immune cell function. Harvested blood serum from burn-injured mice was incubated with bone marrow and splenocytes derived from healthy, untreated mice. Serum from vehicle-treated scalded mice resulted in necrosis of ∼50, ∼40, and ∼25% in $CD4^+$, $CD8^+$, and monocytes/macrophages, respectively, similar to historic controls in mouse splenocytes serum cocultures (15). Serum from TPPU-treated mice showed significantly less necrosis in all three cell types in a dose-dependent manner (Fig. 2).

EpOME Decreases Apoptosis of Nonstimulated Memory T Cells But Has Little Effect on Myeloid Apoptosis and Cell Compartment Acidification. We assessed the effect of EpOME on innate and adaptive immune cells. A total of 2 μM EpOME was administered to innate and adaptive immune cells under stimulated and nonstimulated conditions. The 9,10-EpOME was selected because this compound has been associated with increased ARDS severity in sepsis patients and in animal models of sepsis (11, 16). The stimulation of the spleen cell suspension with CD3 revealed it promotes $CD4^+$ and $CD8^+$ T cell apoptosis (Fig. 3 *A* and *C*). In contrast to the general T cell population, CD3 stimulation reduced the induction of apoptosis in the memory T cell subpopulations (Fig. 3 *B* and *D*), which are defined as CD62L-low–expressing T cells (17). The incubation with 9,10-EpOME did not alter apoptosis induction in either stimulated or nonstimulated $CD4^+$ and $CD8^+$ T cell populations (Fig. 3 *A* and *C*), but 9,10-EpOME reduced it in their nonstimulated CD62L-low subpopulations (Fig. 3 *B* and *D*).

The stimulation of myeloid cells with lipopolysaccharide (LPS) increased apoptosis in neutrophils, defined as $Ly6G^+Ly6^{+/-}$ (Fig. 3*E*) but decreased it in monocytes and macrophages, defined as $Ly6C^+Ly6G^-$ cells (Fig. 3*G*). Moreover, the acidification of cell compartments such as phagosomes and lysosomes was assessed in these cell types, which revealed that LPS induces acidification in both neutrophils and monocytes (Fig. 3 *F* and *H*). The incubation of the spleen cell suspension with EpOME reduced apoptosis in stimulated neutrophils to a small but statistically significant extent (Fig. 3*E*) and reduced the capacity to acidify monocyte and macrophage cell compartments (Fig. 3*H*).

DiHOME Impairs Innate Immune Cell Functionality by Driving Maturation and Activation in Stimulated Neutrophils, Impairing Monocyte and Macrophage Functionality, and Reducing Cytokines Produced by Myeloids. Given that DiHOMEs increase in murine burn injury (Fig. 1), we examined what effect 12,13-DiHOME would exert on innate immune cells. 12,13-DiHOME was selected because it has been identified to have the largest increase in severe COVID-19 disease, and it also is involved in brown fat adipogenesis (7, 10). Amplified expression of CD11b and ICAM-1 on neutrophils mirrors neutrophil activation and maturation (18), because it is associated with an increase in phagocytic capacity and reactive oxygen species (ROS) production (18, 19). We found the in vitro incubation of nonstimulated neutrophils from a spleen cell suspension showed decreased expression of CD11b (Fig. 4*B*) and ICAM-1 (Fig. 4*C*). However, under stimulation with interferon gamma (IFNγ) (Fig. 4*A*) and LPS (Fig. 4 *B* and *C*), both CD11b (Fig. 4 *A* and *B*) and ICAM-1 (Fig. 4*C*) expression increased when stimulated with DiHOME. Next, we found the ability of monocytes and macrophages in a spleen cell suspension to acidify internal cell compartments impaired by DiHOME under nonstimulated conditions (Fig. 4*D*). The expression of major histocompatibility complex class II (MHC-II), which is

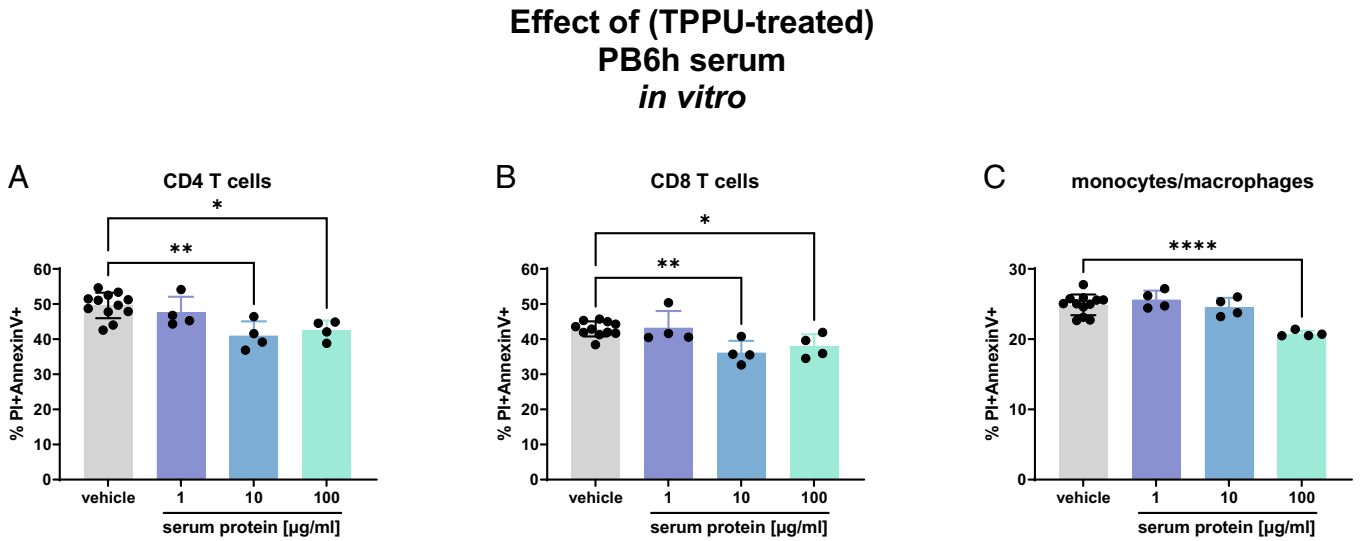

Fig. 2. TPPU prevents immune cell necrosis. Male C57BL/6N mice were divided into four groups (n = 4 to 6/group). The mice underwent the same burn and TPPU/vehicle treatment as described above (Fig. 1) with the only difference that TPPU/vehicle was administered twice at 24 h and directly before burn injury and not three times. Serum from all mice was harvested 6 h postburn injury (PB6H) and pooled within the groups. Cell suspensions from spleen (*A* and *B*) and bone marrow (*C*) from a male C57BL/6N mouse were then incubated for 24 h with increasing amounts of serum proteins; burn-injured mice were treated with TPPU. Controls were treated with serum of vehicle-treated mice. After an incubation of 24 h the cell suspensions were harvested and labeled for flow cytometry to identify CD4 and CD8 T cells, as well as monocytes and macrophages. To assess whether incubation with the respective serum prevents necrosis, cells were stained with PI and Annexin V and analyzed via flow cytometry. For plotting of the data, cells treated with serum of vehicle-treated mice were combined as there were no differences among the different levels of serum proteins. All cells treated with increased protein concentrations from serum of mice, which did not receive TPPU treatment, were combined in the analysis and marked as one vehicle group. Data were compared using a one-way ANOVA test and a P value of <0.05 was considered statistically significant. Data are presented as mean ± SD. *P < 0.05, **P < 0.01, ****P < 0.0001.

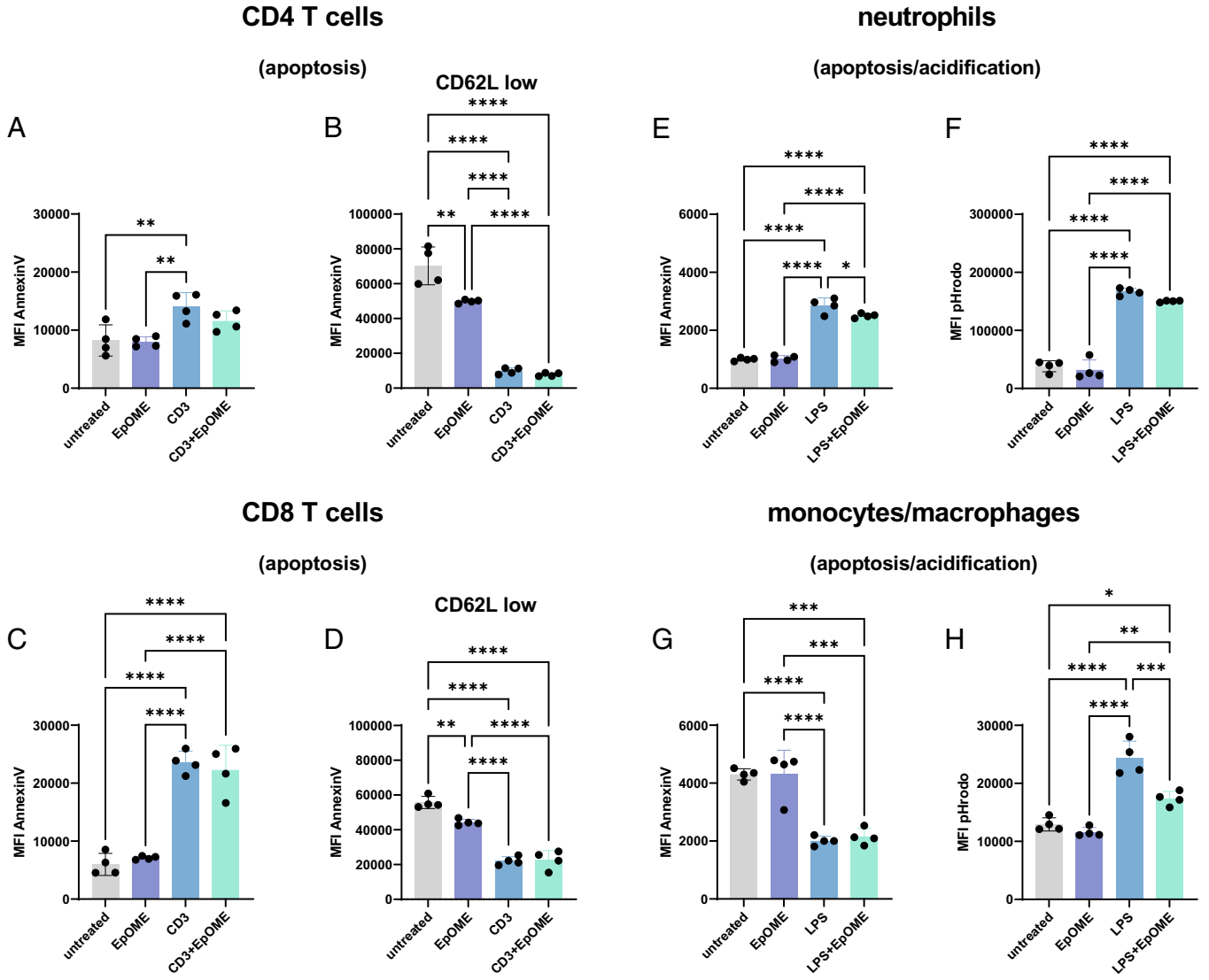

Fig. 3. EpOME has little effect on apoptosis of CD4, CD8 T cells, and myeloid cell apoptosis and acidification. (*A–D*) Spleen cell suspension was treated with or without 9,10-EpOME and plate-bound CD3 for 24 h in cell culture media. The cells were then harvested, stained, and analyzed via flow cytometry to identify CD4 (*A* and *B*) and CD8 (*C* and *D*) T cells and gated for their respective CD62L^{low} subpopulation (*B* and *D*), which excludes the naïve T cell populations. (*E–H*) To assess the effect of EpOME on myeloid cells, bone marrow from untreated mice was harvested with or without 9,10-EpOME and LPS for 24 h in vitro. Via flow cytometry, neutrophils (*E* and *F*) and monocytes and macrophages (*G* and *H*) were identified. (*A–E* and *G*) To examine whether treatment with CD3, LPS, or 9,10-EpOME increases cell apoptosis, cells were labeled with Annexin V and the MFI was measured. (*F* and *H*) In addition to apoptosis, we analyzed the acidification of internal cell compartments in the phagocyting myeloid cells. Bone marrow cell suspension was exposed and incubated with particles of *E. coli* labeled with pHrodo dye, which become increasingly fluorescent with decreasing pH value when phagocyted into neutrophils, monocytes, or macrophages. (*A–H*) All groups in all experiments (n = 4/group) were compared using one-way ANOVA and a P value of <0.05 was considered statistically significant. Data are presented as mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

associated with immunosuppressive functionality (20) tended to be impaired by DiHOME under nonstimulated conditions and was found significantly impaired under stimulated conditions (Fig. 4*E*). Last, we assessed the concentrations of IL-6 and TNF-α in the supernatants of the spleen cell suspensions. All myeloid cell types are known to be potent producers of IL-6 and TNF-α, when stimulated (21–23). When treated with 12,13-DiHOME, both cytokine concentrations were found to be significantly reduced in the supernatants (Fig. 4 *F* and *G*).

Discussion

Burn injury in mice and humans is characterized immunologically by an acute increase (<24 h) of absolute numbers of innate immune cells, whereas lymphocyte numbers drop and mainly proinflammatory cytokines such as TNF-α, IL-6, and IL-1β are released systemically (24, 25). Studies focused on innate immune functions reveal an impairment of innate immune cells in severe burn outcomes because neutrophils show both reduced ROS production and ability to phagocyte pathogens (25), and monocytes display reduced bactericidal activity (26). Kosaka et al. found increased EpOME levels associated with mortality in burn patients (11); however, few studies have examined the correlation between the concentrations of DiHOMEs and outcome in burn patients. Hamaguchi et al. conducted one of the few studies investigating DiHOME levels in burn patients (27). In that case study, only five patients were analyzed (four survivors and one fatal case) with the fatal case showing significantly increased DiHOMEs compared to survivors. We therefore assessed the effect of EpOME and DiHOME on innate and adaptive immune cells, with the aim of examining whether the dysfunction in these cells in burn injury can be attributed to the effect of these oxylipins.

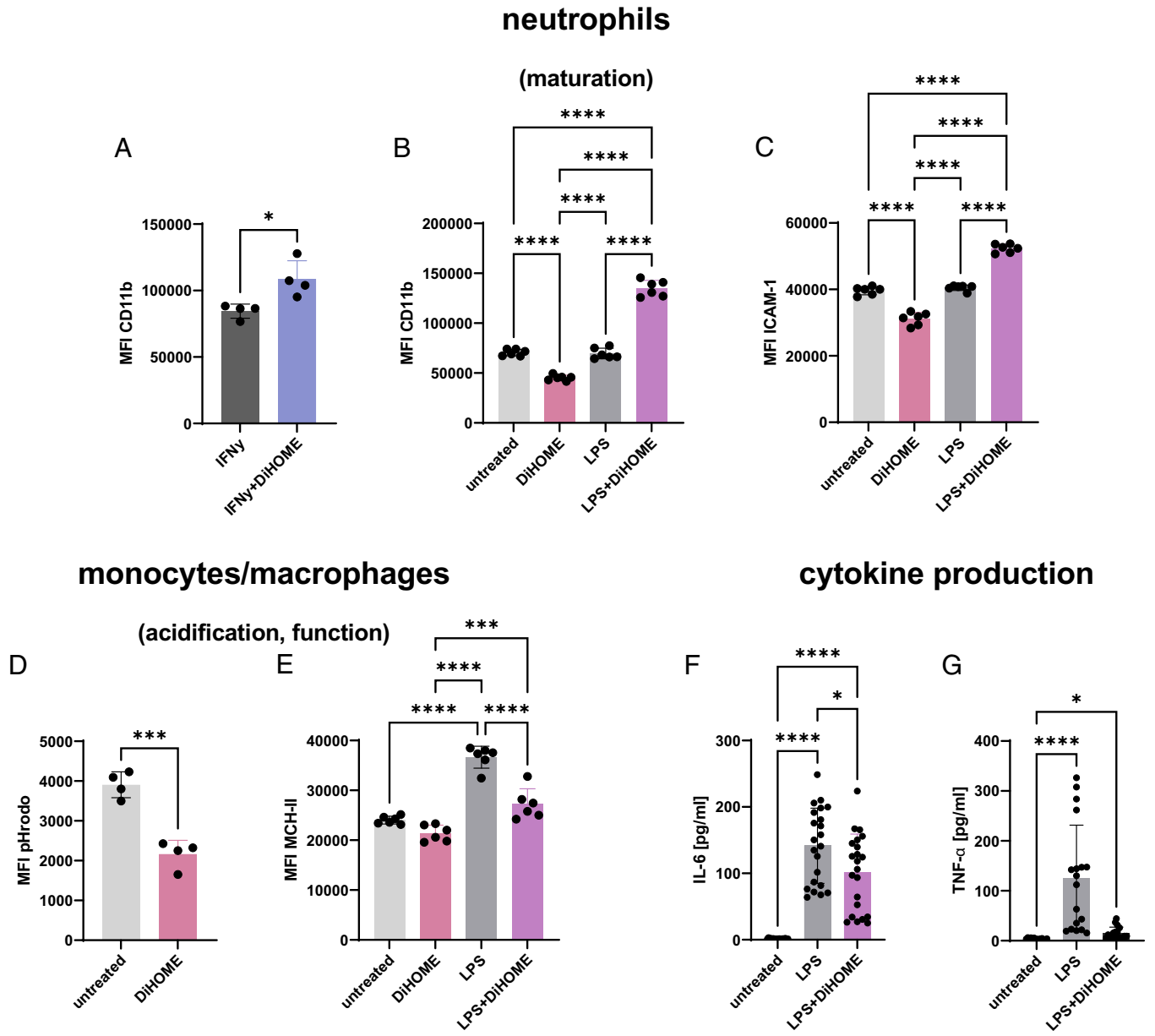

Fig. 4. DiHOME impairs multiple innate immune cell functions. (*A*–*G*) In order to examine the effect of DiHOME on myeloid cell functionality, we stimulated spleen cell suspensions with either IFNγ, LPS, or 12,13-DiHOME for 24 h in cell culture media. (*A*–*E*) The cells were then harvested and labeled for functional and phenotypical analysis. (*F* and *G*) Moreover, we collected the supernatants of the cell suspensions and measured their IL-6 and TNF-α concentrations, both of which are potently produced by myeloid cells. (*A*–*C*) Neutrophil markers CD11b and ICAM-1 (CD54) were assessed because increased expression mirrors maturation and activation of neutrophils. (*D*) The cell suspension was exposed and incubated with *E. coli* opsonized pHrodo dye that increases its fluorescence intensity when phagocyted and with declining pH values, and thereby serves as a marker for the acidification of internal cell compartments such as the lysosomes. (*E*) Monocyte and macrophage MHC-II expression was measured to assess shifts toward a functionally immunosuppressive type that expresses decreased levels of MHC-II. (*F* and *G*) Myeloid cells are potent producers of IL-6 and TNF-α when stimulated; therefore, their concentrations in the spleen cell suspension supernatants were analyzed to reveal impaired production mirroring decreased myeloid ability to produce proinflammatory cytokines when stimulated. (*A* and *D*) Groups ($n = 4$) were compared using a nonpaired Student's *t* test. (*B*, *C*, and *E*) All groups analyzing cells ($n = 6$) and cytokine concentrations (*F* and *G*) ($n = 18$ to 22/per group, distributed over five different experiments) were tested for statistically significant differences using a one-way ANOVA test. A *P* value of <0.05 was considered statistically significant. Data are presented as mean ± SD. **P* > 0.05, ****P* > 0.001, *****P* > 0.0001.

EpOMEs are metabolized rapidly to DiHOMEs by sEH (10). First, we measured systemic oxylipin levels 24 h postburn and found DiHOME levels significantly increased (Fig. 1). Then we assessed whether they can be therapeutically reduced. TPPU, a soluble sEH inhibitor, decreased 9,10- and 12,13-DiHOME 24 h postburn injury (Fig. 1). Moreover, we found TPPU-treated serum from burn-injured mice harvested 6 h postburn decreased necrosis of adaptive and innate immune cells in a dose-dependent manner (Fig. 2). Given the detrimental effect of lymphopenia in inflammation (28), we believe that the prevention of DiHOME formation via TPPU diminished adaptive and innate immune dysfunction in burn injury, and we further examined the effects of EpOME and DiHOME on the innate immune response.

Studying adaptive immunity, we found 9,10-EpOME has no effect on the induction of apoptosis in either stimulated or nonstimulated $CD4^+$ and $CD8^+$ T cell populations (Fig. 3 *A* and *C*). We further examined the memory T cell subpopulation, defined as CD62L-low–expressing T cells (17), because they more potently contribute to the immune response by producing IFNγ as an example (29, 30). EpOME reduced apoptosis in unstimulated memory T cells (Fig. 4 *B* and *D*). In burn

injury, $CD4^+$ T cell apoptosis is induced over time (31), and this apoptosis-driven lymphopenia was revealed to be associated with a detrimental outcome in other inflammatory conditions such as sepsis, where the experimental prevention of apoptotic death of lymphocytes was shown to increase murine survival (28). We therefore conclude that our data do not support the assumption of Kosaka et al., that increased mortality can be attributed to elevated EpOME levels (11). Because some epoxides other than 1,2-disubstituted fatty acid epoxides are highly reactive chemically, biological activity is often attributed to these three-membered cyclic ethers rather than to the generally nonreactive diol metabolites. Kosaka et al. did not monitor DiHOME concentrations in their published study (11). We assume that the correlation of high EpOME levels and adverse outcome in their data can be attributed to increased DiHOME instead of EpOME, because our data show an increase in DiHOME levels in the burn-injured mice compared to controls (Fig. 1*B*), and previously cited in vitro work shows pathology largely can be attributed to the DiHOMEs and not the EpOMEs (8, 9).

Further, the examination of neutrophils did not support previous assumptions from Kosaka et al. (11) that the mortality was due to the EpOMEs, because we found that the EpOME-treated cells show slightly reduced signs of apoptosis (Fig. 3*E*). Reduced neutrophil apoptosis might benefit the host in burn injury as it was shown that neutrophils become less apoptotic after burn (32), potentially contributing to hyperinflammation. However, given the small quantitative effect observed in our samples, this remains speculative until further experimental examination. The EpOME did reduce acidification of stimulated monocytes and macrophages (Fig. 3*H*). The acidification of phagolysosome is necessary for monocyte phagocytosis (33), which is impaired postburn (26). These findings suggest that EpOME might contribute to some innate immune cell dysfunction postburn, most likely only in specific cells such as monocytes. We therefore conclude that our data do not support the assumption of Kosaka et al. that increased mortality can be attributed to elevated EpOME levels (11); rather, these effects appear largely due to the DiHOME metabolites Kosaka et al. did not monitor. We hypothesize that the correlation they reported can be attributed to increased DiHOME levels. In support of this hypothesis, our data show an increase in DiHOME levels in the burn-injured mice compared to sham (Fig. 1). To further examine whether innate immune dysfunction can be attributed to either EpOMEs or DiHOMEs, we assessed the effect of DiHOME on innate immunity.

The incubation of splenic neutrophils with 12,13-DiHOME increased neutrophil maturation under stimulated conditions (Fig. 4 *A*–*C*), but decreased it under nonstimulated conditions (Fig. 4 *B* and *C*). The release of damage-associated molecular patterns and pathogen-associated molecular patterns creates a highly inflammatory stimulus in severely burn-injured organisms as a protective mechanism against passage of pathogenic bacteria through the impaired skin or gut barriers or the respiratory tract (34). Therefore, we tested the effect of 12,13-DiHOME on neutrophils with two different stimuli, IFNγ and LPS, both known to prime neutrophils (35). Incubation with 12,13-DiHOME increased the expression of both CD11b and ICAM-1 to a greater degree than LPS or IFNγ alone, which reflect maturation and activation of neutrophils and increased ROS production (18, 36). It is not yet fully understood whether this promotion of neutrophil maturation is beneficial or leads to neutrophil exhaustion in burn injury (14). Several studies showed that neutrophils posttrauma express high CD11b surface levels but are unable to further increase it when stimulated (37, 38). We therefore interpret high neutrophil CD11b expression as a sign of neutrophils reaching maximum activation but without the ability to further combat subsequent immunological assault, such as infection or major surgery.

Next, we found that monocyte acidification and MHC-II expression is diminished by 12,13-DiHOME (Fig. 4 *D* and *E*), which decreases their ability to phagocytize pathogens (33). Diminished expression of monocyte human leukocyte antigen-DR isotype (HLA-DR), which is a MHC-II isotype, was found in several burn studies and is associated with mortality and the development of sepsis (39). Decreased HLA-DR expression on monocytes under highly inflammatory conditions was shown to be associated with an antiinflammatory cytokine profile (40). An important function of splenic macrophages following burn injuries is the production of proinflammatory cytokines, such as IL-6 and TNF-α (41). The in vitro treatment of the splenic cell suspension with 12,13-DiHOME decreased IL-6 and TNF-α production significantly after LPS stimulation (Fig. 4 *F* and *G*). It is of note that we did not isolate the monocyte population; therefore, IL-6 and TNF-α production cannot be directly attributed to only monocytes. However, given that LPS, a strong myeloid stimulant, specifically induces TNF-α production in monocytes and neutrophils but not macrophages (42) or T cells, we can assume that the effect largely is monocyte and neutrophil dependent. This suggests that 12,13-DiHOME impairs a key neutrophilic and monocytic function, leaving them dysfunctional and the host susceptible to secondary infection, a common cause of morbidity and mortality in burn patients. In addition, we have previously reported that DiHOME causes massive alveolar edema and hemorrhage in mice (9).

We assessed whether systemic DiHOME levels can be therapeutically decreased and found that TPPU, a selective sEH inhibitor, significantly decreased 12,13-DiHOME and nonsignificantly decreased 9,10-DiHOME 24 h postburn injury (Fig. 1). Moreover, we found that TPPU-treated serum from burn-injured mice harvested 6 h postburn decreased necrosis of adaptive and innate immune cells in a dose-dependent manner (Fig. 2). Given the detrimental effect of lymphopenia in inflammation and critical illness, as discussed above, we believe sEH inhibitors such as TPPU are suitable therapeutic agents to diminish adaptive and innate immune dysfunction in burn injury by preventing DiHOME formation. Previous studies determined the specificity of both omega-3 and -6 fatty acid epoxides for the sEH enzyme and also determined that the LA epoxides had the highest affinity, demonstrated by the lowest K_m, of all epoxide regioisomers from different PUFA lipids tested with the exception of a few lipids (*cis* and *trans* 9,10-EpO, γ-12,13-EpODE, and 11,12-EET). Furthermore, sEH metabolizes the 12,13-EpOME slightly faster than the 9,10-EpOME; however, the lack of statistical significance in decreasing 9,10-DiHOME after TPPU treatment can be explained by non-sEH hydrolysis by the structurally and catalytic very different epoxide hydrolase enzyme, microsomal epoxide hydrolase, which can hydrolyze the 9,10- but not the 12,13-EpOME and not as efficiently as sEH (43). This is consistent with the oxylipin profile showing elevated DiHOMEs from burned mice, suggesting higher activity of the sEH enzyme in response to burn and the importance of inhibiting the formation of these metabolites. Furthermore, animals with higher omega-3 fatty acid epoxides demonstrate an antiinflammatory phenotype; however, the biological activity of the omega-3 diols has not been investigated (44). Thus, it is uncertain whether the

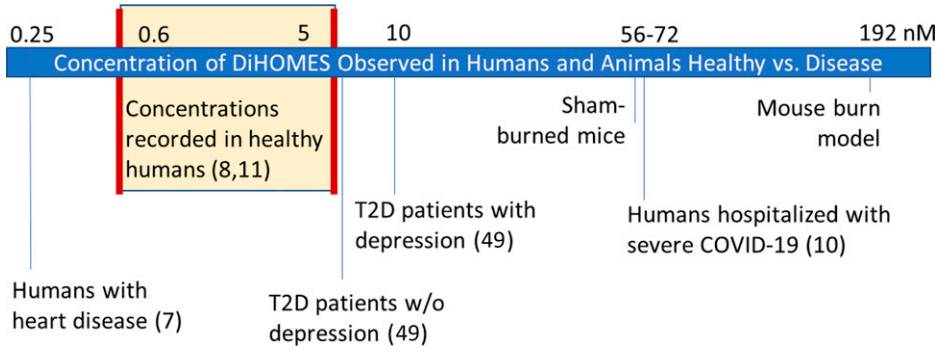

Fig. 5. DiHOME concentrations reported in human disease and animal models indicate a disease-specific and/or concentration-dependent effect on beneficial or deleterious outcomes. Pinckard et al. characterized decreased 12,13-DiHOME concentration in patients with cardiac disease compared to healthy human controls, concluding a protective effect of this oxylipin (7). Pinckard et al. also characterized function of this adipokine in a mouse cardiac model to demonstrate that brown fat is cardioprotective and that 12,13-DiHOME maintains brown fat adipogenesis. Anita et al. (49) characterized fatty acid profiles in patients with type 2 diabetes (T2D) with and without depression and found that diabetic patients have increased DiHOMEs that are even more pronounced in diabetic patients with depression. McReynolds et al. demonstrated increased DiHOMES in severe human COVID patients (10). Finally, normal mice display higher DiHOMEs than humans [62 nM calculated in sham-treated mice from Bergmann et al. (12) and the current study], but the concentrations of DiHOMES observed in this mouse study exceeded those concentrations observed in other diseases in humans and therefore further studies are needed to characterize DiHOME levels in burn patients and correlate them to the level of EpOMEs observed in severe and septic burn cases (11).

antiinflammatory profile is a result of the increased epoxides or decreased inflammatory diols. The current study investigates the biological activity of the epoxides and diols of LA. Although it may be unrealistic to monitor the biological activity of the entire fatty acid metabolism cascade in each study, care should be taken in interpreting past studies that did not explore the activity of the full metabolic pathway.

DiHOMEs demonstrate a wide variety of biological effects, having both deleterious and beneficial implications depending on tissue concentration and disease conditions. The AHA recommendation to increase PUFA consumption resulted in part from observational studies showing improved health in humans with high LA consumption (1), and the understanding that LA is an essential PUFA derived entirely from dietary intake (5). Furthermore, recent studies demonstrate that decreased concentrations of DiHOMEs are associated with poor outcomes in patients with heart failure compared to healthy, age-matched patients. Part of the beneficial cardiovascular effects may be attributed to increased brown fat adipogenesis associated with DiHOMEs (7). This function has further beneficial effects by regulating thermogenesis and improving insulin sensitivity in patients with metabolic syndrome. In accordance with these activities, DiHOMEs (particularly the 12,13- molecule) are sometimes referred to as adipokines or batokines. In these conditions, DiHOMEs are driving what appears to be a beneficial effect; however, the effects reported in disease conditions indicate the benefits of DiHOMEs are observed at low concentrations, while higher concentrations are associated with negative and inflammatory outcomes, such as ARDS, COVID-19, and sepsis (Fig. 5).

In summary we conclude that there is a baseline level of DiHOMEs required for the important biological function of brown fat adipogenesis; however, highly increased levels of DiHOME impair key innate immune functions that have significant implications in dietary recommendations. Our data suggest that DiHOMEs rather than EpOMEs are a key driver of immune dysfunction in burn injury by indicating that DiHOMEs, and not EpOMEs, support a phenotype of hyperinflammatory neutrophilic response. In these experiments, the biologically relevant regioisomers were used to probe the hypothesis that the DiHOMEs are responsible for driving increased mortality in burn: the 9,10-EpOME was tested as the regioisomer that significantly increased in response to burn, and 12,13-DiHOME was tested as the regioisomer that responded to sEH inhibition. Recent literature revealed an inflammatory pattern of hyperinflammatory neutrophils, reflected by increased CD11b expression, in combination with dysfunctional and suppressive monocytes, reflected by decreased HLA-DR expression, in burn injury (13, 14), trauma (37), and bacterial (45) and viral (46) sepsis, which is associated with detrimental outcomes. The same patterns can be functionally seen in our data when innate cells were treated with DiHOME (Fig. 4). Future studies will benefit from assessing the effects of both regioisomers of EpOMEs and DiHOMEs on immune function. Furthermore, we provide a rationale to further explore the mechanistic effects of the impairment of adaptive and innate immunity and for the therapeutic use of sEH inhibitors in murine models potentially translated and applied to humans in the future.

Materials and Methods

Animal Models. Male and female C57BL/6N and male outbred CD1 IGS mice, both 8 to 12 wk old, were purchased from Charles River. Female C57BL/6N mice were used for in vitro analyses and outbred CD1 IGS mice, as well as male C57BL/6N mice, for the in vivo trauma models. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Cincinnati (IACUC protocol no: 08-09-19-01) in accordance with all institutional and federal guidelines, and reporting in the manuscript follows the recommendations in the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines (47).

Burn Injury Model and Administration of TPPU. Male C57BL/6N mice underwent scald injury after being shaved on their back and placed in a plastic mold exposing 28% of the total body surface area (TBSA) under inhalation anesthesia with 4% isoflurane in oxygen. The exposed back was then subjected to 90 °C hot water for 9 s, resulting in a third-degree burn injury of the respective area (48). Afterward the mice were resuscitated with 1 mL of 0.9% normal saline injected i.p. and placed on a 42 °C heating pad for 3 h. As a control, mice underwent sham treatment by exposure to room temperature water. To assess the effect of TPPU, a group of mice received 10 mg/kg body weight TPPU (300 μg per mouse, i.p.) (Cayman Chemical) diluted in 100% polyethylene glycol (PEG) with an average molecular weight of 400 Da (Sigma-Aldrich) at 48 h, 24 h, and directly before induction of burn injury. The control group ($n = 4$) was administered PEG. The final three groups consisted of sham treatment without TPPU administration ($n = 4$) and burn injury with TPPU ($n = 6$) and with vehicle (PEG) ($n = 6$) treatment. All mice were euthanized after 24 h and blood serum was collected to analyze systemic oxylipin levels via mass spectrometry.

To evaluate the effect of blood serum from burn injury, TPPU ($n = 4$) or vehicle-treated ($n = 4$) male C57BL/6N mice underwent the exact same burn

injury procedure as described above, different only in receiving two treatments of TPPU or vehicle, 24 h and directly before injury induction. Blood serum was harvested 6 h postinjury induction, for in vitro incubation with innate and adaptive immune cells.

Mass Spectrometry. Whole blood was collected 24 h postburn injury, maintained at room temperature for 30 min, and centrifuged at 4 °C to gain blood serum. Immediately after serum collection, an antioxidant solution (0.2% triphenylphosphine/0.2% butylated hydroxytoluene/0.1% ethylenediaminetetraacetic acid per 100 μL of serum) was added at 2% of volume and frozen at −80 °C until analysis. For analysis of lipid profiles, nine isotope-labeled oxylipins were added to the samples as internal controls at 3× volume in methanol and extracted using solid phase extraction followed by the liquid chromatography-electrospray ionization/multistage mass spectrometry UPLC/MS/MS system (Waters Acquity UPLC) and the AB Sciex 6500+ QTrap system as previously described (12).

In Vitro Incubation with EpOME and DiHOME. The effect of EpOME and DiHOME treatment in spleen and bone marrow cell suspensions was evaluated by incubating 2 million cells of the respective cell suspension for 24 h at 37 °C in a cell culture media consisting of RPMI (BioWhittaker, Lonza Group AG), gentamycin, minimum essential medium, sodium pyruvate, penicillin-streptomycin-glutamine (all purchased from Gibco, Thermo Fisher Scientific), and Cytiva HyClone fetal bovine serum (Thermo Fisher Scientific). After euthanasia, bone marrow was harvested by flushing the tibia and femur of both legs, and spleens were harvested by splenectomy and gently mashed through a 70-μm mesh (Falcon Cell Strainers, Thermo Fisher Scientific) to gain a cell suspension. All in vitro experiments were conducted in 24-well plates with 2 million cells per well. Untreated cells contained only cell media. T cell stimulation was performed by adding 1 μg/mL CD3e (BD Pharmingen) in phosphate buffered saline (Sigma-Aldrich) per well, which was then incubated for 1 h so the CD3e became plate bound. Then 9,10-EpOME was added to a final concentration of 2 μM to evaluate its effect on T cell behavior. Myeloid cells were primed by adding 100 ng/mL LPS (*Escherichia coli* 0111:B4, Sigma-Aldrich) into the cell media to the respective cells. Moreover, a final concentration of 2 μM 9,10-EpOME or 5 μM 12,13-DiHOME was achieved in the respective wells to evaluate their effect on myeloid cell behavior. The spleen cell suspension stimulation with IFNγ required the addition of 2.5 ng/mL IFNγ (Sigma-Aldrich) with or without 5 μM 12,13-DiHOME, all in cell culture media.

In Vitro Incubation with Serum from TPPU-Treated, Burn-Injured Mice. Serum from male C57BL/6N mice was harvested 6 h postburn or postsham intervention with or without TPPU administration, as described above. The sera from scalded mice treated with TPPU ($n = 4$) and without TPPU treatment ($n = 4$) were pooled for ex vivo treatment of splenocytes and bone marrow cells. The amount of protein in the pooled sera was assessed using a BCA Protein Assay Kit (Pierce Protein Biology, Thermo Fisher Scientific) according to the manufacturer's instructions. Spleen and bone marrow cell suspensions were harvested from one naïve male C57BL/6N mouse. These suspensions were incubated in cell culture media with the pooled sera from the scald-injured and TPPU-treated mice containing 1, 10, or 100 μg/mL protein for 24 h at 37 °C. The same incubation was performed with the pooled sera from the scald-injured mice not treated with TPPU. For the analysis, the latter sera with all three protein concentrations were combined. The cells were then harvested and labeled for flow cytometry identification of $CD4^+$ and $CD8^+$ T cells, neutrophils, monocytes, and macrophages, and for the assessment of necrosis, both as described below.

Acidification Assay (pHrodo Assay). According to the manufacturer's instructions we processed opsonized peptides from *E. coli* labeled with pH-sensitive dyes (pHrodo Green BioParticles from *E. coli*; Thermo Fisher) and subsequently incubated the spleen cell suspensions with them at 37 °C, 5% CO_2 for 1 h. This allowed neutrophils, monocytes, and macrophages to phagocytize these particles. Phagocytosis was then interrupted by placing the cells on ice and fixing them with 1% paraformaldehyde (PFA). For the remainder of the processing, cells were kept on ice and subsequently labeled for flow cytometry analysis as described below. Internal cell compartments such as phagosomes and lysosomes become acidified after the digestion of the phagocyted particles, which correlates with the increased intensity of the light signal of the pH-sensitive dyes, reflected in its mean fluorescence intensity (MFI) in flow cytometry. A decrease in the MFI was assumed to reflect a decrease in the ability to acidify internal cell compartments.

Flow Cytometry and Assessment of Apoptosis and Necrosis. The harvested cells were enumerated using a cell counter (Beckman Coulter) and 1 million cells were washed and afterward labeled for flow cytometry. First a Fc-receptor blockage was conducted by incubating the cells with CD16/CD32 (Mouse BD Fc Block) (clone 2.4G2 [RUO], BD Pharmingen) and 5% rat serum (Invitrogen) for 10 min at room temperature. Consecutively, the cells were labeled with the antibodies for 20 min at 4 °C in the dark. The following fluorescent-labeled antibodies were used: Ly6G (clone: 1A-8), Ly6C (clone: AL-21), CD4 (clone: RM4-5), ICAM-1(CD54) (clone: 3E2), L-Selectin (CD62L) (clone: MEL-14), CD11b (clone: M1/70), MHC-II (clone: AF6-120.1) (all from BD Biosciences) and CD8 (clone: 53-6.7) (BioLegend). The analysis of the labeled cells was performed on an Attune NxT Acoustic Focusing Cytometer (Thermo Fisher Scientific).

To evaluate the share of apoptotic or necrotic cells, they were labeled with Annexin V (BD Pharmingen) and/or propidium iodide (PI) (Fluka Chemie GmbH). After harvesting, cells were washed and resuspended in Annexin V Buffer (BD Biosciences), counted, and diluted into 100 μL buffer containing 1×10^5 cells. Each sample was then incubated with 5 μL Annexin V antibody and 1 μL 100 μg/mL and/or PI for 15 min. Consecutively, the samples were washed, placed on ice, labeled, and analyzed via flow cytometry as described above. Cells were indicated to become increasingly apoptotic when showing increasing Annexin V MFI values. Cells that were found to be Annexin V and PI positive were assumed necrotic.

Cytokines. To assess cell supernatant cytokine concentrations, the spleen cell suspensions were centrifuged and supernatants collected after a 24-h incubation time. The concentrations of IL-6 and TNF-α were measured using the BD Cytometric Bead Array Mouse IL-6 Flex Set and Mouse TNF Flex Set (BD Biosciences) according to the manufacturer's instructions.

Statistical Analysis. The statistical analysis was performed with GraphPad Prism 9.1.2 (GraphPad Software). All groups were tested for normality using the Shapiro–Wilk test (method of Royston) and the D'Agostino and Pearson normality test omnibus K2. If groups were normally distributed, a two-tailed Student's *t* test comparison of two groups (Fig. 4 *A* and *D*) or one-way (Fig. 2) or two-way (Fig. 1) ANOVA with Tukey (Fig. 2) or of Benjamini, Krieger, and Yekutieli (Fig. 1) post hoc analysis for comparisons of more than two groups was applied (Figs. 1, 3, and 4 *B*, *C*, *E*, and *F*). If groups were not normally distributed, a Mann–Whitney test to compare two groups or Kruskal–Wallis test with Dunn's multiple-comparison test analysis for comparisons of more than two groups was conducted (Fig. 3*G*). Data are expressed as the mean ± SD. A P value of ≤ 0.05 was considered statistically significant.

Data Availability. All study data are included in the article and/or *SI Appendix*. Study data is also available at https://doi.org/10.25338/B8QP99.

ACKNOWLEDGMENTS. We thank Holly Goetzman for her great help conducting the experiments and assistance with the surgeries. This work was supported by funding from the Deutsche Forschungsgemeinschaft (German Research Foundation) BE 7016/1-1 (C.B.B.), Shriners Hospitals for Children Grant 85800-CIN-17 (D.M.S.), NIH/National Institute of General Medical Sciences Grant T32GM113770 (to C.B.M.), NIH/National Heart, Lung, and Blood Institute Grant T32HL086350 (N.S.), NIH/National Institute of Environmental Health Sciences (NIEHS) Grant R35 ES030443-01 (RIVER Award) (B.D.H.), and NIH/NIEHS Superfund Program Grant P42 ES004699 (B.D.H.).

[a]Author affiliations: Division of Research, Department of Surgery, College of Medicine, University of Cincinnati, Cincinnati, OH 45221; [b]Department of Entomology and Nematology, University of California, Davis, CA 95616; [c]Division of Plastic, Reconstructive, and Hand Surgery/Burn Surgery, Department of Surgery, University of Cincinnati College of Medicine, Cincinnati, OH 45229; [d]Scientific Staff, Shriners Children's Ohio, Dayton, OH 45404; and [e]UCD Comprehensive Cancer Center, University of California, Sacramento, CA 95817

Author contributions: C.C.C., D.M.S., and B.D.H. designed research; C.B.B., D.W., and H.G. performed research; C.B.B. and C.B.M. analyzed data; and C.B.B., C.B.M., and N.S. wrote the paper.

1. D. Kritchevsky, History of recommendations to the public about dietary fat. *J. Nutr.* **128** (suppl.), 449S–452S (1998).
2. A. Borsini *et al.*, Omega-3 polyunsaturated fatty acids protect against inflammation through production of LOX and CYP450 lipid mediators: Relevance for major depression and for human hippocampal neurogenesis. *Mol. Psychiatry* **26**, 6773–6788 (2021).
3. D. Swanson, R. Block, S. A. Mousa, Omega-3 fatty acids EPA and DHA: Health benefits throughout life. *Adv. Nutr.* **3**, 1–7 (2012).
4. I. de Bus, R. Witkamp, H. Zuilhof, B. Albada, M. Balvers, The role of n-3 PUFA-derived fatty acid derivatives and their oxygenated metabolites in the modulation of inflammation. *Prostaglandins Other Lipid Mediat.* **144**, 106351 (2019).
5. A. A. Spector, Essentiality of fatty acids. *Lipids* **34** (suppl.), S1–S3 (1999).
6. K. Hildreth, S. D. Kodani, B. D. Hammock, L. Zhao, Cytochrome P450-derived linoleic acid metabolites EpOMEs and DiHOMEs: A review of recent studies. *J. Nutr. Biochem.* **86**, 108484 (2020).
7. K. M. Pinckard *et al.*, A novel endocrine role for the BAT-released lipokine 12,13-diHOME to mediate cardiac function. *Circulation* **143**, 145–159 (2021).
8. M. F. Moghaddam *et al.*, Bioactivation of leukotoxins to their toxic diols by epoxide hydrolase. *Nat. Med.* **3**, 562–566 (1997).
9. J. Zheng, C. G. Plopper, J. Lakritz, D. H. Storms, B. D. Hammock, Leukotoxin-diol: A putative toxic mediator involved in acute respiratory distress syndrome. *Am. J. Respir. Cell Mol. Biol.* **25**, 434–438 (2001).
10. C. B. McReynolds *et al.*, Plasma linoleate diols are potential biomarkers for severe COVID-19 infections. *Front. Physiol.* **12**, 663869 (2021).
11. K. Kosaka, K. Suzuki, M. Hayakawa, S. Sugiyama, T. Ozawa, Leukotoxin, a linoleate epoxide: Its implication in the late death of patients with extensive burns. *Mol. Cell. Biochem.* **139**, 141–148 (1994).
12. C. B. Bergmann *et al.*, TPPU treatment of burned mice dampens inflammation and generation of bioactive DHET which impairs neutrophil function. *Sci. Rep.* **11**, 16555 (2021).
13. H. Moins-Teisserenc *et al.*, Severe altered immune status after burn injury is associated with bacterial infection and septic shock. *Front. Immunol.* **12**, 586195 (2021).
14. J. Johansson, F. Sjogren, M. Bodelsson, F. Sjoberg, Dynamics of leukocyte receptors after severe burns: An exploratory study. *Burns* **37**, 227–233 (2011).
15. H. M. Kim *et al.*, Facilitation of apoptosis by autologous serum and related immunosuppression in the splenocyte culture. *Immunopharmacology* **34**, 39–50 (1996).
16. T. Ozawa *et al.*, Biosynthesis of leukotoxin, 9,10-epoxy-12 octadecenoate, by leukocytes in lung lavages of rat after exposure to hyperoxia. *Biochem. Biophys. Res. Commun.* **134**, 1071–1078 (1986).
17. V. Golubovskaya, L. Wu, Different subsets of T cells, memory, effector functions, and CAR-T immunotherapy. *Cancers (Basel)* **8**, 36 (2016).
18. S. Sengupta, C. C. Caldwell, V. Nomellini, Distinct neutrophil populations in the spleen during PICS. *Front. Immunol.* **11**, 804 (2020).
19. R. van Bruggen *et al.*, Complement receptor 3, not Dectin-1, is the major receptor on human neutrophils for beta-glucan-bearing particles. *Mol. Immunol.* **47**, 575–581 (2009).
20. A. E. Mengos, D. A. Gastineau, M. P. Gustafson, The $CD14^{+}HLA\text{-}DR^{lo/neg}$ monocyte: An immunosuppressive phenotype that restrains responses to cancer immunotherapy. *Front. Immunol.* **10**, 1147 (2019).
21. M. Rossol *et al.*, LPS-induced cytokine production in human monocytes and macrophages. *Crit. Rev. Immunol.* **31**, 379–446 (2011).
22. Z. Zhang *et al.*, CEACAM1 regulates the IL-6 mediated fever response to LPS through the RP105 receptor in murine monocytes. *BMC Immunol.* **20**, 7 (2019).
23. N. C. Riedemann *et al.*, Regulatory role of C5a in LPS-induced IL-6 production by neutrophils during sepsis. *FASEB J.* **18**, 370–372 (2004).
24. F. Zhang *et al.*, Burn-related dysregulation of inflammation and immunity in experimental and clinical studies. *J. Burn Care Res.* **38**, e892–e899 (2017).
25. P. Hampson *et al.*, Neutrophil dysfunction, immature granulocytes, and cell-free DNA are early biomarkers of sepsis in burn-injured patients: A prospective observational cohort study. *Ann. Surg.* **265**, 1241–1249 (2017).
26. M. Kobayashi *et al.*, M2b monocytes predominated in peripheral blood of severely burned patients. *J. Immunol.* **185**, 7174–7179 (2010).
27. M. Hamaguchi *et al.*, A case series of the dynamics of lipid mediators in patients with sepsis. *Acute Med. Surg.* **6**, 413–418 (2019).
28. R. S. Hotchkiss *et al.*, Prevention of lymphocyte cell death in sepsis improves survival in mice. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 14541–14546 (1999).
29. P. S. de Araújo-Souza *et al.*, Differential interferon-γ production by naive and memory-like CD8 T cells. *J. Leukoc. Biol.* **108**, 1329–1337 (2020).
30. S. F. Yu, Y. N. Zhang, B. Y. Yang, C. Y. Wu, Human memory, but not naive, CD4+ T cells expressing transcription factor T-bet might drive rapid cytokine production. *J. Biol. Chem.* **289**, 35561–35569 (2014).
31. J. Patenaude, M. D'Elia, C. Hamelin, D. Garrel, J. Bernier, Burn injury induces a change in T cell homeostasis affecting preferentially CD4+ T cells. *J. Leukoc. Biol.* **77**, 141–150 (2005).
32. Z. Hu, M. M. Sayeed, Suppression of mitochondria-dependent neutrophil apoptosis with thermal injury. *Am. J. Physiol. Cell Physiol.* **286**, C170–C178 (2004).
33. E. Uribe-Querol, C. Rosales, Phagocytosis: Our current understanding of a universal biological process. *Front. Immunol.* **11**, 1066 (2020).
34. M. G. Jeschke *et al.*, Burn injury. *Nat. Rev. Dis. Primers* **6**, 11 (2020).
35. P. P. McDonald, C. Bovolenta, M. A. Cassatella, Activation of distinct transcription factors in neutrophils by bacterial LPS, interferon-gamma, and GM-CSF and the necessity to overcome the action of endogenous proteases. *Biochemistry* **37**, 13165–13173 (1998).
36. S. Takizawa, A. Murao, M. Ochani, M. Aziz, P. Wang, Frontline science: Extracellular CIRP generates a proinflammatory $Ly6G^{+}$ $CD11b^{hi}$ subset of low-density neutrophils in sepsis. *J. Leukoc. Biol.* **109**, 1019–1032 (2021).
37. J. Hazeldine *et al.*, Prehospital immune responses and development of multiple organ dysfunction syndrome following traumatic injury: A prospective cohort study. *PLoS Med.* **14**, e1002338 (2017).
38. D. A. Rodeberg, R. C. Bass, J. W. Alexander, G. D. Warden, G. F. Babcock, Neutrophils from burn patients are unable to increase the expression of CD11b/CD18 in response to inflammatory stimuli. *J. Leukoc. Biol.* **61**, 575–582 (1997).
39. F. Venet *et al.*, Decreased monocyte human leukocyte antigen-DR expression after severe burn injury: Correlation with severity and secondary septic shock. *Crit. Care Med.* **35**, 1910–1917 (2007).
40. A. Fabri *et al.*, Characterization of circulating IL-10-producing cells in septic shock patients: A proof of concept study. *Front. Immunol.* **11**, 615009 (2021).
41. M. G. Schwacha, M. W. Knöferl, I. H. Chaudry, Does burn wound excision after thermal injury attenuate subsequent macrophage hyperactivity and immunosuppression? *Shock* **14**, 623–628 (2000).
42. V. Seow *et al.*, Inflammatory responses induced by lipopolysaccharide are amplified in primary human monocytes but suppressed in macrophages by complement protein C5a. *J. Immunol.* **191**, 4308–4316 (2013).
43. C. Morisseau *et al.*, Relative importance of soluble and microsomal epoxide hydrolases for the hydrolysis of epoxy-fatty acids in human tissues. *Int. J. Mol. Sci.* **22**, 4993 (2021).
44. C. López-Vicario *et al.*, Inhibition of soluble epoxide hydrolase modulates inflammation and autophagy in obese adipose tissue and liver: Role for omega-3 epoxides. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 536–541 (2015).
45. G. P. Leijte *et al.*, Monocytic HLA-DR expression kinetics in septic shock patients with different pathogens, sites of infection and adverse outcomes. *Crit. Care* **24**, 110 (2020).
46. Z. Parackova *et al.*, Disharmonic inflammatory signatures in COVID-19: Augmented neutrophils' but impaired monocytes' and dendritic cells' responsiveness. *Cells* **9**, 2206 (2020).
47. N. Percie du Sert *et al.*, Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biol.* **18**, e3000411 (2020).
48. J. Tschöp *et al.*, Differential immunological phenotypes are exhibited after scald and flame burns. *Shock* **31**, 157–163 (2009).
49. N. Z. Anita *et al.*, Serum soluble epoxide hydrolase related oxylipins and major depression in patients with type 2 diabetes. *Psychoneuroendocrinology* **126**, 105149 (2021).