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Lactic acid suppresses IgE-mediated mast cell function *in vitro* and *in vivo*

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ABSTRACT

Mast cells have functional plasticity affected by their tissue microenvironment, which greatly impacts their inflammatory responses. Because lactic acid (LA) is abundant in inflamed tissues and tumors, we investigated how it affects mast cell function. Using IgE-mediated activation as a model system, we found that LA suppressed inflammatory cytokine production and degranulation in mouse peritoneal mast cells, data that were confirmed with human skin mast cells. In mouse peritoneal mast cells, LA-mediated cytokine suppression was dependent on pH- and monocarboxylic transporter-1 expression. Additionally, LA reduced IgE-induced Syk, Btk, and ERK phosphorylation, key signals eliciting inflammation. *In vivo*, LA injection reduced IgE-mediated hypothermia in mice undergoing passive systemic anaphylaxis. Our data suggest that LA may serve as a feedback inhibitor that limits mast cell-mediated inflammation.

1. Introduction

Mast cells function as innate sentinels, rapidly responding to bacterial, viral, and parasitic pathogens [1]. However, their detrimental response to allergens is central to allergic disease [2,3]. While mast cells are activated by a variety of stimuli, the best characterized is IgE-antigen crosslinking of the high affinity IgE receptor, FcεRI, which elicits a biphasic response [4]. The early phase is characterized by degranulation, releasing a myriad of pre-formed mediators, including histamine, proteases, proteoglycans, and some cytokines [5]. This is rapidly followed by release of inflammatory lipids, including arachidonic acid metabolites and sphingosine-1-phosphate [6,7]. The late phase consists of *de novo* cytokine and chemokine synthesis, which can dictate the migration and function of other cell populations [8,9]. These responses are collectively inflammatory. However, anti-inflammatory and pro-angiogenic activities also occur, including synthesis and activation of latent TGFβ1, and angiogenesis induced by VEGF, tryptase, and chymase [10–18].

Mast cell function is altered by tissue microenvironmental cues, including growth factors, cytokines, and chemokines. These stimuli can influence IgE-induced activation. For example, SCF augments FcεRI signals [19], while this pathway is blunted by TGFβ1 [20,21]. Recent interest in immunometabolism prompted us to investigate how the glycolytic by-product LA affects mast cell function. LA concentrations vary widely with metabolic activity and tissue perfusion, and correlate with clinical features. For example, LA or lactate levels are linked to outcomes or severity in cancer [22–25], sepsis [26], pulmonary embolism [27], and asthma [2,28,29]. But more than just a marker of glucose metabolism, LA is functionally important. Lactate or LA suppresses the inflammatory functions of macrophages, dendritic cells, and T cells [30–36].

We recently found that LA suppresses mast cell responses to IL-33 [37]. The current work extends this to IgE-mediated mast cell function. Our results show that LA suppresses FcεRI phosphorylation signals, correlating with reduced degranulation and the production of inflammatory cytokines and chemokines. Furthermore, LA suppressed IgE-mediated anaphylactic shock *in vivo*, supporting our contention that this metabolic product has immunomodulatory effects that include feedback suppression of IgE-induced inflammation. These data shed new light on how metabolism is tied to allergic inflammation, an area of growing importance.

Abbreviations: BMMC, bone marrow derived mast cells; DNP-HAS, dinitrophenyl-coupled human serum albumin; LA, lactic acid; MCT-1, monocarboxylic transporter-1; PMC, peritoneal mast cells; PSA, passive systemic anaphylaxis; SCF, stem cell factor; SkMC, human skin mast cells

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2. Materials and Methods

2.1. Animals

C57BL/6J male and female mice were purchased from The Jackson Laboratory (Bar Harbor, ME), bred in the VCU Animal Care Facility, and used at a minimum of 6 weeks old, with approval from the Virginia Commonwealth University Institutional Animal Care and Use Committee (IACUC).

2.2. Mouse mast cell cultures

Mouse peritoneal mast cells (PMC) were obtained by collecting peritoneal lavage from C57BL/6J mice. Cells were expanded in complete RPMI (cRPMI) 1640 medium (Invitrogen Life Technologies, Carlsbad, CA) containing 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM sodium pyruvate, and 1 mM HEPES (all from Corning, Corning, NY), cRPMI supplemented with recombinant mouse IL-3 and SCF at 10 ng/ml each for 14 days, at which time they were approximately 85% mast cells, based on staining for c-Kit and FcεRI surface expression. Bone marrow derived mast cells (BMMC) were derived by harvesting bone marrow from C57BL/6J mouse femurs, followed by culture in cRPMI supplemented with IL-3-containing supernatant from WEHI-3B cells and SCF-containing supernatant from BHK-MKL cells. The final concentrations of IL-3 and SCF were adjusted to 1.5 ng/ml and 15 ng/ml, respectively, as measured by ELISA. BMMC were used after 3 weeks of culture, at which point these primary populations were > 90% mast cells, based on staining for c-Kit and FcεRI surface expression.

2.3. Human skin mast cell culture

As approved by the Internal Review Board at the University of South Carolina, surgical skin samples were collected from the Cooperative Human Tissue Network of the National Cancer Institute. Skin mast cells (SkMC) were harvested and cultured from 5 human donors as previously described [38]. Mast cells were used after 6–10 weeks of culture, when purity was nearly 100%, as confirmed with toluidine blue staining.

2.4. Cytokines and reagents

Recombinant mouse IL-3 and SCF, as well as mouse IL-6, TNF, and MCP-1 (CCL-2) ELISA kits were purchased from BioLegend (San Diego, CA). Mouse MIP-1α (CCL-3) and VEGF ELISA kits were purchased from PeproTech (Rocky Hill, NJ). Mouse IL-13 ELISA kits were purchased from eBioscience (San Diego, CA). L-(+)-lactic acid and Sodium L-lactate were purchased from Sigma-Aldrich (St. Louis, MO). Human TNF and MCP-1 ELISA kits were purchased from BD OptEIA (BD Biosciences; Franklin Lakes, NJ).

2.5. Cell culture conditions

For IgE crosslinking, mouse mast cells were incubated with 0.5 μg/ml of mouse IgE clone C38-2 (BD Pharmingen, San Jose, CA) overnight. SkMC were incubated with 1 μg/ml IgE overnight. Cells were washed and resuspended at 2×10^6 cells/ml in same media growth factors used for culture. An equal volume of 25 mM LA in cRPMI was added to the cell suspension, resulting in a final cell concentration of 1×10^6 cells/ml, 10 ng/ml of IL-3 and SCF, and 12.5 mM LA. Control conditions received cRPMI in place of LA. After 24 h of pretreatment in LA media, cells received 50 ng/ml of DNP-HSA for 16 h, after which supernatants were collected. pH was measured for media alone, lactic acid-conditioned media, and lactate-conditioned media using a Beckman Phi 45 pH meter.

2.6. Degranulation assay

Mast cells were cultured with IgE (0.5 μg/ml) overnight, then treated ± 12.5 mM LA for 24 h as described in Section 2.5. IL-3 and SCF were removed during the last 4 h prior to activation. Cells were activated with DNP-HSA (50 ng/ml) for 10 min at 37 °C, then washed twice with PBS and stained for degranulation using PE anti-CD63 and APC anti-CD107a (BioLegend, San Diego, CA), using a BD FACSCelesta (BD Biosciences, San Jose, CA).

2.7. RT-qPCR assay

TRIzol was purchased from Life Sciences (Grand Island, NY) and used to extract total RNA. Nucleic acid purity was measured using a Nanodrop 1000 UV–Vis Spectrophotometer (Thermo Scientific, Waltham, MA). cDNA was synthesized with the qScript cDNA synthesis kit (Quanta Biosciences, Gaithersburg, MD) following the manufacturer's protocol. To determine IL-6 and GAPDH mRNA expression, real time quantitative PCR (RT-qPCR) was performed with the CFX96 Touch Real-Time PCR Detection System (Bio-Rad) and PerfeCTa SYBR Green SuperMix (Quanta Biosciences). Primers for IL-6 (forward: 5′-TCCAGTTGCCTTCTTGGGAC-3′, reverse: 5′-GTGTAATTAAGCTCCG ACTTG-3′) and GAPDH (GAPDH forward, 5′-GATGACATCAAGAAGGT GGTG-3′, reverse, 5′-GCTGTAGCCAAATTCGTTGTC-3′) were purchased from Eurofins MWG Operon (Huntsville, AL). Amplification conditions were as follows: 95 °C (2 min) followed by 40 cycles of 95 °C (15 s), 55 °C (30 s), and 60 °C (1 min). A melting curve analysis was performed between 50 °C and 95 °C. Results were normalized to GAPDH and the H_2O control by using the Relative Livak Method (ΔΔCt).

2.8. Surface staining with flow cytometry

PMC were cultured ± IgE (0.5 μg/ml) overnight, then treated ± 12.5 mM LA for 24 h as in Section 2.5. Cells were stained with a PE-anti IgE antibody to determine if LA exposure affected surface staining. Cells cultured without IgE were stained with APC-anti-FcεRIα to determine if LA altered FcεRI surface expression. Samples were analyzed with a FACSCelesta flow cytometer (BD Biosciences, San Jose, CA).

2.9. PhosFlow

PMC were cultured ± IgE (0.5 μg/ml) overnight, then treated ± 12.5 mM LA for 24 h as in Section 2.5. IL-3 and SCF were removed during the last 4 h of culture. Cells were activated with DNP-HSA (50 ng/ml) for 10 min at 37 °C, then fixed with 1.6% paraformaldehyde. For permeabilization, cells were resuspended in PBS and slowly added to ice-cold methanol solution while vortexing. Cells were then washed and stained with APC-labeled antibodies against pERK1/2, pSyk, and pBtk and analyzed by flow cytometry using a FACSCelesta.

2.10. Passive systemic anaphylaxis

Age-matched C57BL/6J mice (12 week old mice) were first injected intraperitoneally with anti-DNP IgE (50 μg). 24 h later, all mice received subcutaneous injection of 1 mg/kg ketoprofen (Spectrum Chemical, New Brunswick, NJ), and 30 min later received intraperitoneal injection of either 4 mg/kg LA, given as a 4% (w/v) solution dissolved in PBS or PBS alone. 24 h after LA and PBS injections, all mice received intraperitoneal DNP-HSA (100 μg) to induce anaphylaxis. For histamine-induced anaphylaxis, mice were given the same ketoprofen and LA injections, followed by an intraperitoneal injection of histamine (8 mg) 24 h after LA injection. Core body temperature was measured using a rectal thermometer probe (Physitemp Instruments, Clifton, NJ). Mice were then euthanized via CO_2 asphyxiation. Blood was collected by cardiac puncture in EDTA-coated tubes and

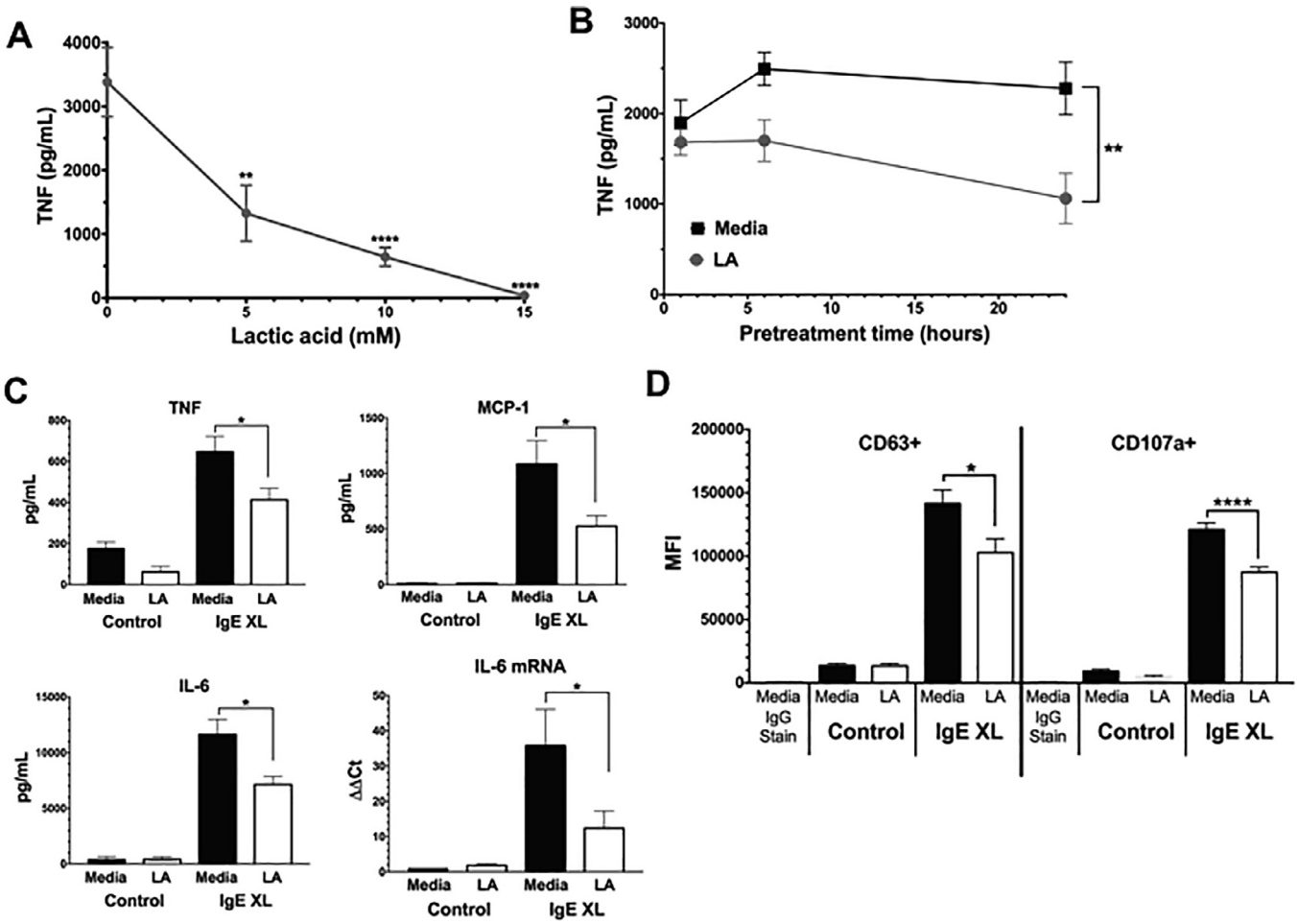

Fig. 1. Kinetics of LA effects on PMC. (A) PMC were pretreated with the indicated concentrations of lactic acid for 24 h prior to IgE-Ag crosslinking (IgE XL). Supernatants were analyzed by ELISA 16 h after activation. Data are from 5 PMC populations analyzed in duplicate. (B) PMC were treated with 12.5 mM LA as in (A), with LA given for the indicated times prior to IgE XL. Data are N = 6 from 2 independent experiments (C) PMC were pretreated with 12.5 mM lactic acid for 24 h prior to IgE-Ag crosslinking for 16 h (ELISA) or 4 h (RT-qPCR). ELISA results are from 10 to 12 samples from 2 independent experiments. RT-qPCR data are from 3 to 4 samples/point. (D) PMC were treated as in (C), with IgE XL for 10 min. CD63 and CD107a expression was measured by flow cytometry. Results are representative of three independent experiments using 3 BMMC populations each, and expressed as mean ± SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; NS, not significant.

centrifuged to isolate plasma.

2.11. Statistical analysis

Data are presented as mean ± SE and analyzed using GraphPad Prism 6 software (GraphPad, La Jolla, CA). Comparisons between two groups were done using unpaired Student's *t* test. Comparisons between multiple groups were done using one-way analysis of variance with Tukey's post-hoc test. All *p* values < 0.05 were deemed significant.

3. Results

3.1. LA suppresses IgE-mediated cytokine production and degranulation by peritoneal mast cells

We first examined LA effects using PMC that were expanded *in vitro*. Cells were cultured ± IgE overnight, washed, and treated for 24 h ± LA at various concentrations, then activated with DNP-HSA for 16 h. As shown in Fig. 1A, LA concentrations ≥5 mM greatly reduced TNF secretion. Using 12.5 mM LA, we found that a 24-h culture period consistently yielded suppression (Fig. 1B). Because we found no change in cell viability with LA concentrations at or below 20 mM (Supplemental Fig. 1), 12.5 mM LA for 24 h was used throughout the study. In addition to reducing TNF, LA also suppressed IgE-induced IL-6 and MCP-1 secretion (Fig. 1C). The reduction in IL-6 protein was mirrored by changes in mRNA (Fig. 1C), suggesting LA reduces cytokine transcription or mRNA stability. In addition to cytokines, LA also inhibited IgE-mediated degranulation. As measured by surface staining for CD63 and CD107a expression [39,40], LA decreased staining intensity (Fig. 1D) without altering the percentage of positive cells (Supplemental Fig. 2). Collectively, these date show that LA can suppress both the early and late phases of IgE-induced mast cell function.

3.2. LA suppresses IgE-mediated cytokine and chemokine production from human skin mast cells

To determine if these results were consistent in human mast cells, we tested SkMC from human donors. Since these cells differentiate *in vivo* and are expanded *ex vivo*, they are conceivably one of the most physiologically relevant sources of mast cells. As with PMC, SkMC were sensitized with IgE overnight, washed and treated ± 12.5 mM LA for 24 h, then activated with antigen for 16 h. As shown in Fig. 2, LA greatly reduced IgE-mediated TNF and MCP-1 production, an effect consistent among all donors.

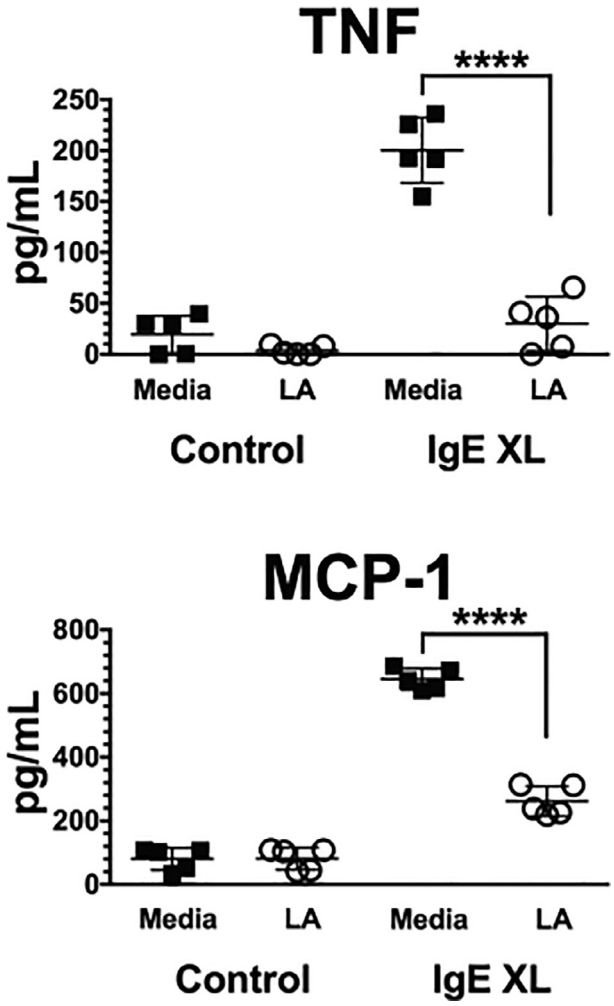

Fig. 2. LA suppresses IgE-mediated cytokine and chemokine production from human SkMC. SkMC from 5 donors were cultured with IgE ± 12.5 mM LA and activated as in Fig. 1C. Supernatants were collected 16 h later and analyzed by ELISA. Each icon represents the mean value of triplicate samples from an individual donor. ****, $p < 0.0001$.

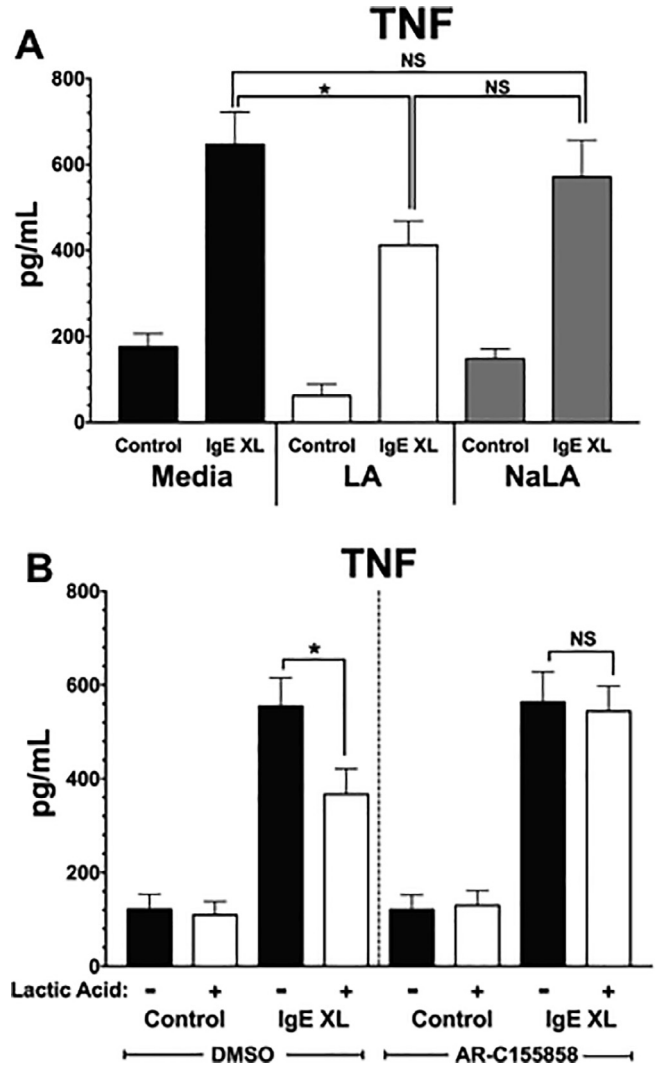

Fig. 3. LA effects are pH- and MCT-1-dependent. (A) PMC were cultured as in Fig. 1C with either 12.5 mM LA or 12.5 mM sodium lactate for 24 h, then activated by IgE XL for 16 h. TNF secretion was measured by ELISA. (B) PMC were pretreated with either an MCT-1/2 inhibitor AR-C155858 (100 nM) or DMSO (vehicle) for 1 h, treated ± 12.5 mM for 24 h, then activated via IgE XL for 16 h. TNF secretion was measured by ELISA. Results are two experiments conducted in triplicate and expressed as mean ± SEM.*, $p < 0.05$; N.S., not significant.

3.3. LA does not alter FcεRIα expression or IgE binding

Since acid pH has been used to strip IgE from FcεRI [41], we determined if LA treatment affected IgE receptor occupancy or FcεRI surface expression. PMC were incubated overnight ± IgE, then with 12.5 mM LA for 24 h. Cells were stained for surface FcεRIα or surface-bound IgE and analyzed via flow cytometry. LA had no effect on either FcεRI surface expression or surface-bound IgE (Supplemental Fig. 3). We conclude that the ability of LA to alter IgE-mediated responses is not due to changes in FcεRI expression or occupancy.

3.4. LA suppresses IgE-mediated cytokine production in a pH- and MCT-1-dependent manner

We recently found that LA suppresses IL-33-induced cytokine production in a pH- and MCT-1-dependent manner [37]. We therefore determined if similar mechanisms explain LA effects on IgE signaling. PMC were incubated overnight with IgE, then treated with either media, 12.5 mM LA, or 12.5 mM sodium lactate (NaLA). After treatment for 24 h, PMC were activated with antigen for 16 h, and supernatants were analyzed for TNF secretion by ELISA. Fig. 3A shows NaLA did not mimic the suppressive effects of LA, indicating that these effects are pH-dependent. To determine if the effect of LA is MCT-1-dependent, PMC were incubated overnight with IgE, then treated with an inhibitor of MCT-1 and MCT-2, AR-C155858. Since we previously found that mast cell do not to express MCT2, any changes with AR-C155858 would be due to MCT-1 blockade [37]. After 1-h incubation with vehicle (DMSO) or AR-C155858, PMC were treated with media or 12.5 mM LA for 24 h, then activated for 16 h with antigen. Fig. 3B shows that AR-C155858 treatment prevented the suppressive effects of LA, since IgE-induced TNF was not altered by LA. We concluded that, as with IL-33, LA suppresses IgE-mediated mast cell activation in a pH- and MCT-1-dependent manner.

3.5. LA suppresses FcεRI signaling

To better understand how LA alters IgE-mediated responses in mast cells, we assessed early events in FcεRI signaling. PMC were incubated overnight with IgE then treated with either media or 12.5 mM LA for 24 h. PMC were then activated with DNP-HSA for 5 min and stained to detected phospho-Syk, phospho-Btk, and phospho-ERK1/2 by flow cytometry analysis. As summarized in Fig. 4, LA pre-treatment reduced all

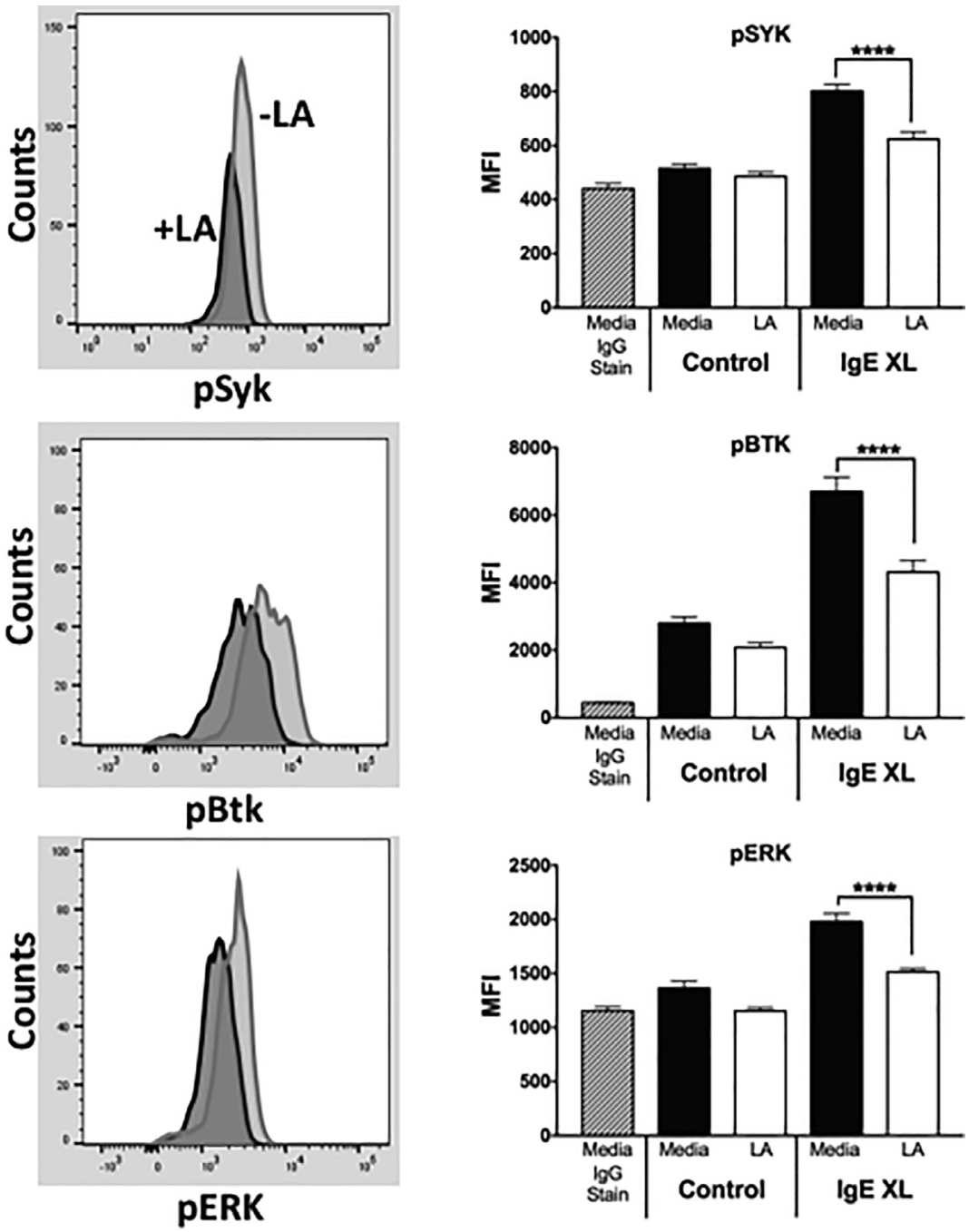

Fig. 4. LA suppresses FcεRI signaling. PMC were given IgE overnight and then treated with 12.5 mM LA for 24 h. During the final 4 h, IL-3 and SCF were removed. Cells were then given Ag for 10 min prior to detection of phospho-proteins as described in Materials and Methods. Left side shows example histograms; right side shows aggregate data. Results are from three experiments conducted in triplicate and expressed as mean ± SEM. ****, $p < 0.0001$.

three phosphorylation events. These data indicate that LA suppresses the activation of key proteins in the IgE signaling cascade.

3.6. LA alters passive systemic anaphylaxis

Lastly, we determined if LA alters IgE-mediated mast cell responses *in vivo*, using the mast cell-mediated PSA model. C57BL/6J mice were injected with IgE, and the next day injected first with ketoprofen to alleviate pain associated with LA, and then either PBS or LA at 4 mg/kg [42]. 24 h after PBS or LA injections, mice were injected with antigen to induce anaphylaxis. As shown in Fig. 5A, LA treatment reduced hypothermia, a key indicator of shock. To determine if LA suppressed vascular histamine responses rather than mast cell function, we tested LA effects on histamine-induced shock. We found that LA treatment did not alter histamine-induced hypothermia (Fig. 5B), indicating that LA is most likely acting on mast cells rather than the vasculature. These data demonstrate that LA can functionally suppress IgE-mediated mast cell responses *in vivo*.

4. Discussion

LA is abundant in blood and tissues, and likely affects cellular function. LA/lactate levels range from 0.5 to 2 mM in healthy tissue, 18 mM in wounds, 25 mM in blood during vigorous exercise, and 40 mM in tumor sites [43,44]. These elevated concentrations could be due to both reduced tissue perfusion and increased glucose metabolism.

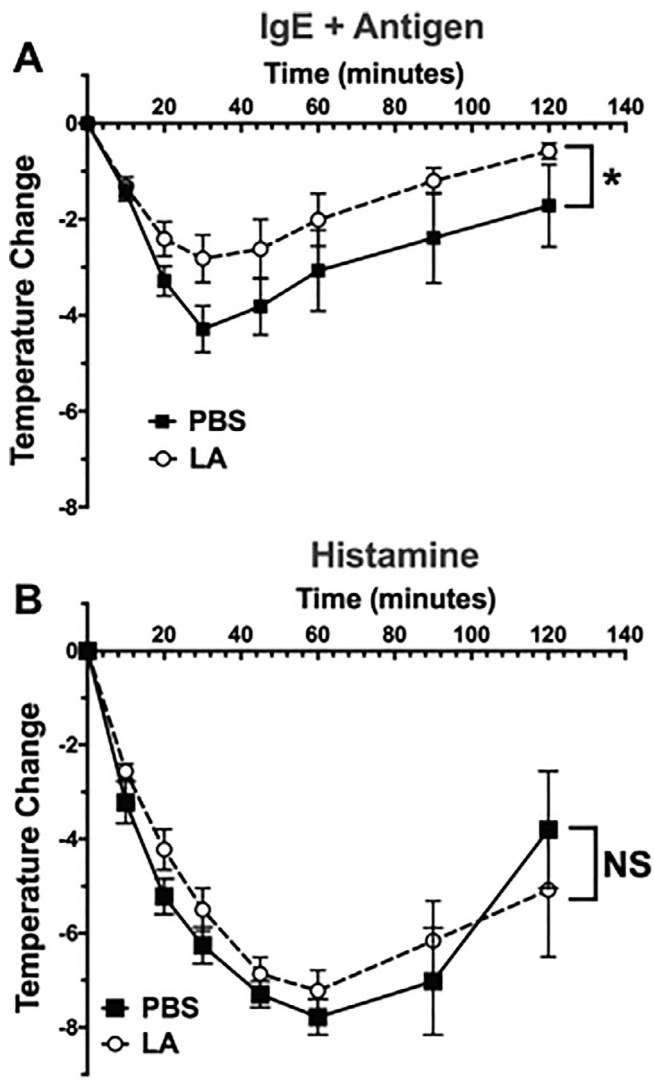

Fig. 5. LA reduces hypothermia in passive systemic anaphylaxis model. (A) Age- and gender-matched C57BL/6J mice were intraperitoneally injected with IgE (50 μg). 24 h later, mice were subcutaneously injected with ketoprofen, and 30 min later intraperitoneally injected with LA or PBS. 24 h later, mice were injected with Ag (100 μg) to induce anaphylaxis. (B) Mice were treated with ketoprofen and LA as in (A), then injected intraperitoneally with 8 mg of histamine. Results shown are from 5 mice per group and expressed as mean ± SEM.*, $p < 0.05$, calculated by Area Under Curve analysis; N.S., not significant.

Therefore, our *in vitro* concentrations are physiologically relevant. Adding 12.5 mM cRPMI-buffered LA to culture media transiently decreased pH to 6.5, which rebounded to 7.2 over 6 h (not shown). These conditions mimic sites of inflammation and were previously shown to suppress mast cell and macrophage function by our lab and others [37,45]. There are no published measures of pulmonary lactic acid concentrations in asthmatics, but patients have acidic exhaled breath condensate (EBC) and elevated serum LA/lactate levels [28,46]. Similarly, systemic anaphylaxis is linked to metabolic acidosis [47–49]. It has not been made clear whether LA/lactate has beneficial or pathological effects on asthma or other mast cell-associated diseases.

In this study, LA suppressed IgE-mediated mast cell function. The mechanism of LA effects is partly apparent from these studies. MCT-1 is a H^+-dependent transporter. We found that both acidity and MCT-1 expression were required for LA effects. We also noted that LA suppressed early events in the FcεRI signaling cascade, including phosphorylation of the critical kinases Syk and BTK. Syk- or BTK-deficient mast cells have defects in IgE-mediated degranulation and cytokine production, suggesting that antagonizing these kinases could explain the suppressive effects noted in PMC and human SkMC. These and other possibilities warrant further investigation. For example, LA uptake could suppress glucose metabolism, which is critical for IgE-mediated mast cell function [50]. Furthermore, IgE signaling in human LAD2 mast cells stabilizes HIF-1α, which is required for IgE-induced TNF and VEGF secretion [37,51]. Because we and others have previously found that LA induces HIF-1 α expression [23,37], this activity should to be at odds with LA suppressing IgE-induced cytokines. This potentially enigmatic role of HIF-1 α is a focus of current studies in our laboratory.

Using 5 human donors distinct from our previous work with IL-33-induced function [37], we found that LA effects were consistent in human skin mast cells. While we have noted donor-to-donor variability among human mast cell responses to statins or TGFβ1 [21,39], all 5 donors responded similarly to LA in this study and in our previous work with IL-33. While too small a group to draw broad conclusions, these data suggest that LA effects are less subject to genetic or other variables.

In summary, this study demonstrates that LA, an abundant by-product of glucose metabolism that is increased in inflammatory environments, suppresses IgE-induced cytokine secretion and degranulation, while enhancing angiogenic effects. The suppressive effects were consistent *in vitro* using mouse or human mast cells, and *in vivo* using an IgE-induced anaphylaxis model. These data indicate that LA can shape the mast cell response, suppressing inflammation while promoting angiogenesis. Understanding the fundamental biology of LA can reveal important aspects of the inflammatory response, especially as this is now appreciated to rely on increased glucose metabolism.

5. Authorship

DA, AJS, HLC, MT, EMK, KNK, TTH, and APC each contributed to data investigation and curation.

DA, SAS, CAO, and JJR each contributed to data conceptualization.

6. Summary statement

Lactic acid inhibits IgE-induced mast cell inflammatory responses *in vitro* and *in vivo*.

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Conflict-of-interest disclosures

The authors have no conflicts of interest related to this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cellimm.2019.04.006.

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