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Time Course Investigation of the Dermal Leukocyte Response to Lipoteichoic Acid in Chickens

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Cover Page Footnote

Ian Gilbert is a May 2022 honors program graduate in Animal Science. Jossie M. Santamaria is a graduate research assistant in Poultry Science. Gisela F. Erf, the faculty mentor, is a professor in the Department of Poultry Science.

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Meet the Student-Author

Ian Gilbert

I am a Summa Cum Laude graduate of spring 2022 with majors in animal science, biology, and Spanish. I grew up in Springdale, Arkansas and graduated from Har-Ber High School. I participated in various organizations throughout my time at the University of Arkansas, including the Bumpers Honors Student Board, Schola Cantorum, Inspirational Chorale, Museum Student Advisory Council, and Pre-Vet Club. Outside of class, I have worked at various veterinary clinics in the area and love spending time with my friends, family, and dog. In the fall, I will be attending veterinary school at Tufts University. I've wanted to be a veterinarian for as long as I can remember, and the strength of the animal science program is what initially drew me to the University of Arkansas. Having a strong interest in animal health, I knew I wanted to conduct research in immunology in order to learn more about how animals combat disease. I've loved being a part of the Bumpers community, and I'm so grateful for everyone I've met. I would like to thank my honors mentor, Dr. Gisela Erf, for her continued guidance. I would also like to thank my committee members Dr. Adnan Alrubaye and Dr. Jeremy Powell. Without these people, this research would not have been possible.

Ian working with graduate student Jossie Santamaria to pluck feathers from chickens which have been injected with lipoteichoic acid.

Research at a Glance

- Lipoteichoic acid (LTA) is a cell-wall component of Gram-positive bacteria which is known to elicit an inflammatory response.
- Intradermal injection of LTA into the pulp of growing feathers of chickens resulted in recruitment of white blood cells, particularly macrophages, from the blood to the site of injection.
- The mechanism underlying the leukocyte infiltration profiles stimulated by LTA in chickens is unclear but differs from other microbial cell-wall products and may be species-dependent.

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Ian M. Gilbert, Jossie M. Santamaria,† and Gisela F. Erf§*

Abstract

Lipoteichoic acid (LTA) is a cell-wall polymer in Gram-positive bacteria that stimulates inflammation. Few studies have investigated in vivo immune response to LTA, and none of the in vivo studies have been performed in birds. For this project, the pulp (a skin derivative) of growing feathers (GF) of chickens was used to investigate the in vivo effects of intradermally injected LTA. In Study 1, GF of chickens were injected with 10 µL of differing concentrations of LTA (0.1, 1.0, 10, 100 or 250 µg LTA/mL; 3 chickens/dose). Growing feathers were plucked before injection (0 h) and at 6, 24, 48, and 72 h post-injection and frozen before staining using immunohistochemistry for visual inspection of leukocyte infiltration. Ten µg/mL LTA was found to be the optimal concentration to stimulate inflammation. In Study 2, GF were injected with 10 µL of either 10 µg/mL LTA (0.1 µg LTA/GF, 12 GF/bird, n = 8) or PBS (vehicle; n = 4). Growing feathers were collected at 0, 6, 24, 48, and 72 h. For each time point, pulp cell suspensions were prepared and immunofluorescently stained with a panel of chicken-leukocyte-specific monoclonal antibodies. Cell populations were analyzed via flow cytometry, revealing elevated levels (% pulp cells) in total leukocytes, monocytes/macrophages, and major histocompatibility complex (MHC) class-II expressing cells in GF injected with LTA when compared to the control. Infiltration of lymphocytes and heterophils was not different between treatment groups. This study suggests that the inflammatory response to LTA in chickens is characterized primarily by recruitment of monocytes/macrophages to the site of inflammation.

* Ian Gilbert is a May 2022 honors program graduate in Animal Science.
† Jossie M. Santamaria is a graduate research assistant in Poultry Science.
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Introduction

The innate immune system is the part of the immune system that consists of the barriers and defenses against infection that the body has without previous exposure (Abbas et al., 2018). Inflammation is an important and complex innate immune response that is still not fully understood. The purpose of this study was to observe in vivo how the inflammatory response initiated by intradermal lipoteichoic acid (LTA) injection progressed over time. Simultaneous intradermal injection of multiple growing feathers (GF) of a chicken with LTA and subsequent periodic sampling of the GF for laboratory analysis provided a profile of the local tissue response to LTA.

The inflammatory response is the first response to injury and infection. During inflammation, vascular changes allow elements of the immune system such as leukocytes and plasma proteins to move from the blood to local tissues at the site of infection (Medzhitov, 2008). These changes are mediated by cytokines released by resident cells in the affected tissue such as macrophages, mast cells, and endothelial cells (Abbas et al., 2018). In the classical inflammatory response, neutrophils, or the analogous heterophils in birds, are initially recruited in high levels, and macrophages are recruited later as inflammation progresses (Abbas et al., 2018). Infections are initially recognized by a group of molecules called pattern recognition receptors on sentinel cells in tissues (i.e., macrophages, dendritic cells, and mast cells) (Günther and Seyfert, 2018). Pattern recognition receptors bind a limited number of molecules that are shared by large groups of pathogens, which signals the cell to respond by activating defenses against infection (Medzhitov, 2008).

Lipoteichoic acid is a cell-wall component of Gram-positive bacteria (Reichmann and Gründling, 2011). It is released during infection and elicits an inflammatory immune response (Ginsburg, 2002). Previous studies have shown that LTA primarily triggers inflammation through activation of toll-like receptor 2 (TLR2) (Oliveira-Nascimento et al., 2012). Other molecules such as the extracellular lipopolysaccharide-binding protein and the membrane bound CD14 and CD36 also play a role in binding LTA and presenting it to TLR2 (Schröder et al., 2004; Oliveira-Nascimento et al., 2012). Toll-like receptor 2 is a membrane bound protein that is expressed in the form of a heterodimer with TLR1 or TLR6 (Abbas et al., 2018). Lipoteichoic acid is able to bind either of the TLR2 heterodimers leading to activation of pro-inflammatory transcription factors NF-κB and AP-1 (Oliveira-Nascimento et al., 2012). In murine macrophages, LTA has also been shown to activate the NLRP6 inflammasome triggering release of inflammatory cytokines and in some cases pyroptosis, inflammatory cell death (Hara et al., 2018).

In vivo studies in mice have shown that intradermal LTA injection results in increased neutrophil and basophil levels based on reverse transcription polymerase chain reaction analysis of mRNA markers (Brauweiler et al., 2019a; Brauweiler et al., 2019b). It is hypothesized that LTA in chickens will trigger inflammatory recruitment of leukocytes to the site of injection. However, which types of leukocytes may be recruited remains unclear.

Materials and Methods

Two groups of male Light-brown Leghorn chickens were raised in floor pens on wood shaving litter with standard light and temperature protocols as described by Shi and Erf (2012). Food and water were provided for ad libitum consumption. This research was approved by the University of Arkansas System Division of Agriculture Institutional Animal Care and Use Committee (UADA-IACUC approval #21035).

For Study 1, the pulp of 18-day-old regenerating GF of 11-week-old chickens was injected with 10 µL of LTA suspension per GF as described in French et al. (2020). Doses of 0.1, 1.0, 10, 100, and 250 µg/mL of LTA (*Staphylococcus aureus*; Sigma-Aldrich, St. Louis, Mo.) in endotoxin-free Dulbecco's phosphate buffered saline (EF-DPBS; Sigma-Aldrich) were administered to 3, 3, 4, 3, and 2 chickens, respectively, with 12 GF injected per bird. Before injection (0 h) and at 6, 24, 48, and 72 hours post-injection (p.i.), GF were collected from each chicken. The pulp of each GF was isolated, placed in optimal cutting temperature freezing medium, flash-frozen in liquid nitrogen, and stored at -80 °C. Frozen, 6-µm thick pulp sections were cut at -23 °C using a cryostat and placed on microscope slides as described in Sullivan and Erf (2017). Blocking buffer, consisting of phosphate buffered saline (PBS; 0.01 M) and 10% horse serum, was added to sections before overnight incubation in a humidified chamber at room temperature. After incubation, slides were washed with PBS and incubated for 30 minutes with a panel of primary mouse-anti-chicken (mac) monoclonal antibodies (mAb). The mac mAbs had specificity for CD45 (pan-leukocyte marker), KUL-01 (macrophage marker), CD3 (pan-T-cell marker), Bu-1 (B-cell marker), CD4 (T-helper-cell marker), CD8 (cytotoxic-T-cell marker), TCR1 (γδ-TCR marker), MHCII (MHC class II expressing cell marker), MCAM (endothelial cell marker), or irrelevant specificity (isotype control). All mAbs were purchased from Southern Biotech (Southern Biotech, Birmingham, Ala.). After incubation, sections were washed with PBS and incubated with biotinylated horse-anti-mouse IgG secondary antibody (Vektor Laboratories, Inc, Burlingame, Calif.). The sections were washed again and incubated 30 minutes with a mixture of avidin and horseradish-peroxidase-

labeled biotin (ABC reagents; Vekta-stain Elite; Vector Laboratories). After washing, peroxide-charged diaminobenzidine tetrahydrochloride (DAB), a colorogenic substrate for the peroxidase, was added to the section. This enzyme-substrate reaction forms a brown precipitate on cells with the antibody-ABC-reagent complexes. The immunochemically stained pulp sections were then counter stained with Methyl Green nuclear stain. Stained tissue sections were observed using a bright field microscope and photographed to visualize leukocyte infiltration to inform decisions on the concentration of LTA to be used in the second part of the study.

In Study 2, the pulp of twelve, 18-day-old GF of 15-week-old chickens were injected with 10 µL of 10 µg/mL LTA suspension (0.1 µg LTA/GF; n = 8) or 10 µL/GF of EF-DPBS (vehicle-control; n = 4). At 0, 6, 24, 48, and 72 hours, a GF was collected from each chicken and placed in Dulbecco's PBS (DPBS) on ice. Pulp suspensions were prepared by incubating pulp in 0.1% collagenase-dispase solution for 10 minutes at 40 °C before being gently pushed through a 60-µm nylon mesh with extra DPBS. Cells were washed twice by centrifugation at 250 × g for 8 minutes at 4 °C, and the final pellet was resuspended in 0.25 mL of PBS+ (0.1 M DPBS, 1% bovine serum albumin, and 0.1% sodium azide). Cell suspensions were stained using a panel of fluorescently labeled mac IgG1 mAbs (Southern Biotech) in two-color and three-color direct staining combinations (French et al., 2020). The first combination used was mac-CD45 mAb conjugated to spectral red (CD45-SPRD) and mac-KUL-01 mAb conjugated to phycoerythrin (KUL-01-PE). The second combination used was mac-TCR1 mAb conjugated to fluoroisothiocyanine (TCR1-FITC; γδ-TCR marker), CD4-PE, and CD8-SPRD. The third combination used was KUL-01-FITC, MHCII-PE, and CD3-SPRD. The final combination was TCR2/TCR3-FITC (αβ-TCR marker) and Bu-1-PE. In a 96-well round-bottom plate, 50 µL of cell suspension was incubated with 50 µL of each staining combination for 30 minutes at 4 °C. Samples of each cell suspension were pooled and incubated with a mixture of fluorescently labeled mac IgG1 mAb of irrelevant specificity to determine non-specific binding and the cut-off between positive and negative fluorescence. Three pools with samples of each cell suspension were single-stained with CD45-FITC, CD45-PE, or CD45-SPRD to set compensation. After incubation, cells were washed twice via centrifugation at 250 × *g* for 4 minutes at 4 °C and resuspended in 200-µL PBS+. Samples were acquired on the flow cytometer (Becton Dickinson Accuri C6 Plus; BD Biosciences, San Jose, Calif.). Flow cytometry data were analyzed using FlowJo software (FlowJo, LLC, Ashland, Ore.) and leukocyte infiltration was expressed as % of total pulp cells in the cell suspension (French et al., 2020).

Using SigmaPlot version 14.5 (Systat Software, Inc, San Jose, Calif.), two-way analysis of variance was conducted to determine the effect of time, treatment, and time by treatment interaction. Multiple means comparisons were made using Fisher's least significant difference (LSD) method. All differences were considered significant at $P \leq 0.05$.

Results and Discussion

Tissue sections from Study 1 showed variation in the extent of leukocyte infiltration in response to varying doses of LTA. There was not an appreciable difference in leukocyte infiltration profiles between 10 µg/mL and 100 µg/mL LTA injection, though at 0.1 µg/mL, 1.0 µg/mL, and 250 µg/mL, overall infiltration seemed to be lower. For this reason, 10 µg/mL was the chosen concentration of LTA to be used in Study 2.

In Study 2, injection of LTA in the GF resulted in pulp-infiltration of leukocytes which reached maximal levels (% of pulp cells) at 6 h p.i. and remained elevated throughout the 72-hour examination (Fig. 1A). Total leukocyte levels were greater in LTA-injected birds at 6, 24, and 72 h compared to the PBS control. Total leukocytes and macrophages were the only populations that showed a time × treatment interaction.

The local cellular response to LTA was dominated by infiltration of macrophages. Levels of macrophages were elevated compared to the control at 24, 48, and 72 h and peaked at 24 h (Fig. 1C). The MHCII-expressing cells also showed a treatment effect with higher levels of expression overall in response to LTA injection (Fig. 1D). As illustrated in Fig. 1, the LTA curves of the percentage of both macrophages and MHCII-expressing cells are very similar, peaking at 24 h p.i. and leveling off at 48 and 72 h at above baseline levels. As macrophages perform antigen presentation and therefore express MHCII, it is likely that this increase in MHCII-expressing cells in response to LTA injection is primarily due to macrophage infiltration.

Heterophil infiltration did not vary significantly between treatments (Fig. 1B). The increase seen at 6 h p.i. is therefore only caused by tissue damage as a result of the injection. Heterophils have been shown to dominate the immune response to intradermal lipopolysaccharide (LPS), a TLR4 ligand found in Gram-negative bacteria (French et al., 2020). Neutrophils, the mammalian equivalent of heterophils, have been described to respond to intradermal LTA in mice (Brauweiler et al., 2019a). Given the results of these two studies, it is surprising that here heterophils were not recruited in response to intradermal LTA. More research needs to be done to investigate how LPS and LTA trigger very different inflammatory responses. There may also be species-specific differences that account for the immune reaction to LTA in chickens and mice.

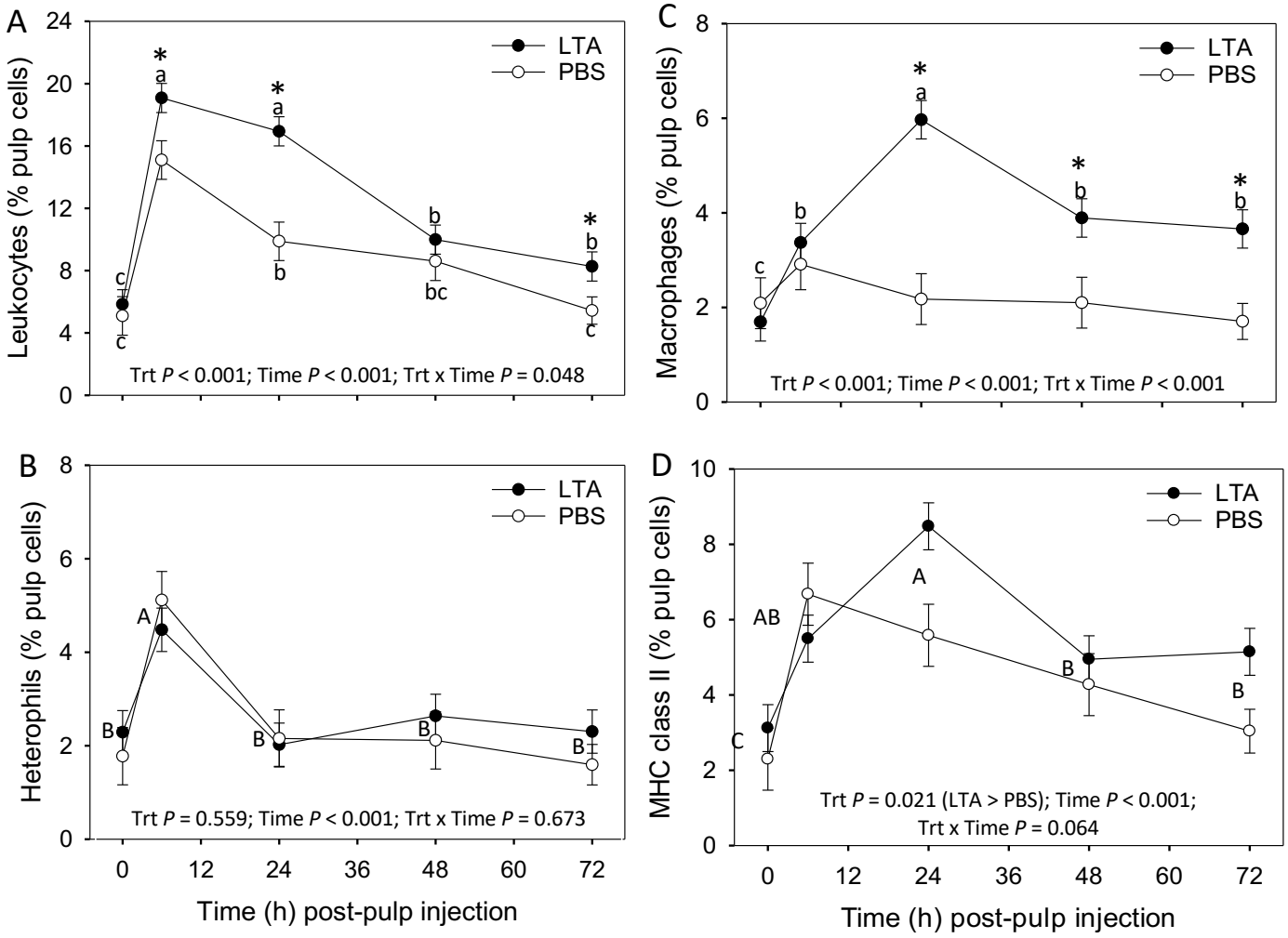

Fig. 1. Total leukocyte (A), heterophil (B), macrophage (C), and major histocompatibility complex class-II expressing pulp cell proportion (D) after intradermal injection of lipoteichoic acid (LTA) or endotoxin-free phosphate buffered saline (PBS; vehicle control) into growing feathers (GF). The GF of 12, 15-week-old chickens were injected with 10 μL PBS (control) or 10 μg/mL of LTA per GF. The GFs were collected before injection (0 hours), and at 6, 24, 48, and 72 hours post injection. Pulp cell suspensions were prepared from GF and immunofluorescently stained using a panel of fluorescence-conjugated mouse monoclonal antibodies against chicken CD45 (pan-leukocyte marker), Kul-01 (macrophage marker), or MHCII. Cell populations were analyzed by flow cytometry; heterophils were identified based on forward and side scatter characteristics of CD45+ cells. Data shown are mean ± SEM; LTA n = 8; PBS n = 4. Within a treatment (Trt), means without a common lowercase letter are different ($P \leq 0.05$); an asterisk (*) indicates Trt means within a time-point are different ($P \leq 0.05$); Time main effect means without a common uppercase letter are different ($P \leq 0.05$).

Lymphocyte populations examined did not vary between treatments (Tables 1 and 2). The ratio of γδ T cells to αβ T cells was calculated and was greater in response to PBS injection (Table 2). However, as γδ T cells and αβ T cells did not differ by treatment, it is unclear what these results mean and why LTA may recruit lower levels of γδ T cells relative to αβ T cells.

Total leukocyte infiltration peaked at 6 h p.i. and was greater in LTA- compared to vehicle-injected GF. However, the only leukocyte subpopulation that showed a time × treatment interaction was macrophages. Macrophages peaked at 24 h p.i. and were not different between treatments at 6 h p.i. This suggests that some other cell population was responsible for the increase in leukocytes in response to LTA injection at 6 h. Brauweiler et al. (2019b) found that LTA stimulated recruitment of basophils in the murine dermis. For this reason, it is possible that basophil infiltration may be responsible for the 6-h peak in LTA-stimulated leukocyte infiltration in birds.

While many studies have investigated in vitro effects of LTA, there is a lack of research investigating the effects of this molecule in vivo. Presented here is the first such in vivo study performed in an avian model. Future research should investigate changes in cytokine and chemokine levels in response to intradermal LTA injection to uncover why LTA-stimulated leukocyte infiltration is dominated by macrophages and lacking in heterophils and lymphocytes. It will also be important to investigate infiltration of other subsets of leukocytes, specifically granulocytes.

Conclusions

The pulp of GF is an established dermal test-site to investigate immune responses taking place in a complex tissue. Intradermal injection of LTA into the pulp of GF was followed by periodic sampling of those feathers and analyses of leukocyte infiltration into the pulp. Intradermal injection of LTA in chickens resulted in infiltration of total

Table 1. Pulp cell proportions of T cells after intradermal injection of lipoteichoic acid (LTA) or phosphate buffered saline (PBS; vehicle control) into growing feathers of 15-week-old chickens.[†]

Treatment	CD3 (%)	CD4 (%)	CD8 (%)	CD4:CD8[‡]
LTA	4.71 ± 0.24[§]	1.45 ± 0.13	2.63 ± 0.10	0.54 ± 0.04
PBS	4.61 ± 0.32	1.42 ± 0.16	2.70 ± 0.13	0.51 ± 0.06
Time (h)				
0	2.67 ± 0.45 c	0.47 ± 0.24 c	2.38 ± 0.19 c	0.19 ± 0.08 d
6	6.28 ± 0.45 a	2.42 ± 0.24 a	3.00 ± 0.19 b	0.80 ± 0.08 a
24	6.05 ± 0.45 ab	1.96 ± 0.24 ab	3.71 ± 0.19 a	0.53 ± 0.08 bc
48	4.89 ± 0.45 b	1.49 ± 0.24 b	2.23 ± 0.19 c	0.67 ± 0.08 ab
72	3.40 ± 0.45 c	0.83 ± 0.20 c	2.01 ± 0.19 c	0.43 ± 0.08 c
Effects (*P*-value)				
Treatment	0.798	0.894	0.687	0.633
Time	<0.001	<0.001	<0.001	<0.001
Treatment x Time	0.470	0.334	0.531	0.529

[†] Growing feathers (GF) of 12, 15-week-old chickens were injected with 10 µL of either PBS (control) or LTA (10 µg/mL) per GF. The GF were collected before injection (0 hours), and at 6, 24, 48, and 72 hours post injection. Pulp cell suspensions were prepared from GF and immunofluorescently stained using a panel of fluorescence-conjugated mouse monoclonal antibodies against chicken CD3 (T cells), CD4 (helper T cells), and CD8 (cytotoxic T cells). Cell populations were analyzed by flow cytometry and data expressed as % of total cells in the pulp cell suspensions.

[‡] CD4:CD8 is the ratio of CD4+ cells to CD8+ cells.

[§] Data shown are mean ± SEM. Means within a column without a common letter are different based on $P \leq 0.050$ based on multiple means comparisons.

leukocytes and macrophages and an increase in MHCII-expressing cells. The macrophage-dominated response to LTA in chickens was unlike the neutrophil- and basophil-dominated response reported in mice and was also different from the heterophil-dominated response to LPS in chickens. This demonstrates the specificity that exists with the immune response to every unique immunogenic substance. Furthermore, it demonstrates the complexity of molecular signaling pathways involved in inflammation. More research is needed to understand why LTA produces a macrophage-dominated inflammatory response in chickens and how that response factors into the overall immune response during a Gram-positive bacterial infection.

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Literature Cited

Abbas, A.K., A.H. Lichtman, and S. Pillai. 2018. Cellular and Molecular Immunology, 9th Edition. Elsevier Saunders, Philadelphia.

Brauweiler, A.M., E. Goleva, and D.Y.M. Leung. 2019a. *Staphylococcus aureus* lipoteichoic acid damages the skin barrier through an IL-1–mediated pathway. J. Investig. Dermatol. 139(8):1753-1761.

Brauweiler, A.M., E. Goleva, and D.Y.M. Leung. 2019b. *Staphylococcus aureus* lipoteichoic acid initiates a TSLP-basophil-IL4 axis in the skin. J. Investig. Dermatol. 140(4):915-917.

French, C.E., M.A. Sales, S.J. Rochell, A. Rodriguez, and G.F. Erf. 2020. Local and systemic inflammatory responses to lipopolysaccharide in broilers: new in-

Table 2. Pulp cell proportions of lymphocytes after intradermal injection of lipoteichoic acid (LTA) or phosphate buffered saline (PBS; vehicle control) into growing feathers of 15-week-old chickens.[†]

Treatment	αβ TCR (%)	γδ TCR (%)	Bu-1 (%)	γδ TCR:αβ TCR[‡]
LTA	3.42 ± 0.18[§]	1.29 ± 0.08	0.57 ± 0.07	0.41 ± 0.02 b
PBS	3.13 ± 0.22	1.47 ± 0.11	0.56 ± 0.09	0.50 ± 0.03 a
Time (h)				
0	1.79 ± 0.33 b	0.88 ± 0.15 b	0.11 ± 0.13 c	0.52 ± 0.05 a
6	4.37 ± 0.33 a	1.91 ± 0.15 a	0.45 ± 0.13 b	0.43 ± 0.05 a
24	3.99 ± 0.33 a	2.06 ± 0.15 a	0.86 ± 0.13 a	0.56 ± 0.05 a
48	3.87 ± 0.33 a	1.02 ± 0.15 b	0.93 ± 0.13 a	0.27 ± 0.05 b
72	2.36 ± 0.27 b	1.04 ± 0.15 b	0.47 ± 0.11 b	0.47 ± 0.05 a
Effects (*P*-value)				
Treatment	0.326	0.192	0.921	0.031
Time	<0.001	<0.001	<0.001	<0.001
Treatment x Time	0.605	0.295	0.104	0.750

[†] Growing feathers (GF) of 12, 15-week-old chickens were injected with either 10 µL PBS (control) or LTA (10 µg/mL) per GF. The GF were collected before injection (0 hours), and at 6, 24, 48, and 72 hours post injection. Pulp cell suspensions were prepared from GF and immunofluorescently stained using a panel of fluorescence-conjugated mouse monoclonal antibodies against chicken αβ T-cell receptor (TCR), γδ TCR, and Bu-1 (B cells). Cell populations were analyzed by flow cytometry and data expressed as % of total cells in the pulp cell suspensions.

[‡] γδ TCR:αβ TCR is the ratio of γδ T cells to αβ T cells.

[§] Data shown are mean ± SEM. Means within a column without a common letter are different based on $P \leq 0.050$ based on multiple means comparisons.

sights using a two-window approach. Poultry Science. 99(22):6593-6605.

Ginsburg, I. 2002. Role of lipoteichoic acid in infection and inflammation. The Lancet Infectious Diseases. 2(3):171-179.

Günther, J. and H.M. Seyfert. 2018. The first line of defence: insights into mechanisms and relevance of phagocytosis in epithelial cells. Seminars in immunopathology. 40(6):555–565.

Hara, H., S.S. Seregin, D. Yang, K. Fukase, M. Chamaillard, E.S. Alnemri, N. Inohara, G.Y. Chen, and G. Núñez. 2018. The NLRP6 inflammasome recognizes lipoteichoic acid and regulates Gram-positive pathogen infection. Cell. 175(6):1651-1664.

Medzhitov, R. 2008. Origin and physiological roles of inflammation. Nature. 454:428-425.

Oliveira-Nascimento, L., P. Massari, and L.M. Wetzler. 2012. The role of TLR2 in infection and immunity. Frontiers Immunol. 3(79).

Reichmann, N.T., and A. Gründling. 2011. Location, synthesis and function of glycolipids and polyglycerolphosphate lipoteichoic acid in gram-positive bacteria of the phylum Firmicutes. FEMS Microbiology Letters. 319(2):97-105.

Schröder, N.W.J., H. Heine, C. Alexander, M. Manukyan, J. Eckert, L. Hamann, U.B. Göbel, and R.R. Schumann. 2004. Lipopolysaccharide binding protein binds to triacylated and diacylated lipopeptides and mediates innate immune responses. J. Immunol. 173(4):2683-2691.

Shi, S., and G.F. Erf. 2012. IFN-γ, IL-21, and IL-10 Co-Expression in Evolving Autoimmune Vitiligo Lesions of Smyth Line Chickens. J. Investig. Dermatol. 132(3):642-649.

Sullivan, K.A. and G.F. Erf. 2017. CD4+ T cells dominate the leukocyte infiltrations response initiated by intradermal injection of phytohemagglutinin into growing feathers in chickens. Poultry Science. 96(10):3574-3580.