A Modulatory Interleukin-10 Response to Staphylococcal Peptidoglycan Prevents Th1/Th17 Adaptive Immunity to Staphylococcus aureus

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Toll-like receptor (TLR) 2 on antigen-presenting cells (APCs) enables these cells to recognize peptidoglycan-embedded lipopeptides and glycopolymers in the Staphylococcus aureus cell wall and mount an inflammatory response to this microbe. TLR2 signalling can also modulate immunity to S. aureus by inducing an interleukin (IL)–10 response in APCs. What determines the balance between proinflammatory and modulatory responses to S. aureus is unknown. We show that the modulatory IL-10 response preferentially occurs upon CD14- and CD36-independent TLR2 signaling, triggering PI3K activation, and is restricted to monocytes and monocyte-derived macrophages (Mφs). In contrast, monocyte-derived dendritic cells (DCs) produce mostly IL-12 and IL-23. The differential APC polarization induced by staphylococcal peptidoglycan translates into differential T helper responses: Mφs primarily trigger IL-10 and weak IL-17 responses, whereas DCs trigger a robust Th1/Th17 response. Exploitation of TLR2 signalling plasticity by S. aureus may explain the wide range of outcomes of human encounters with this microbe.

Staphylococcus aureus is a Gram-positive bacterium present in the nostrils, upper respiratory tract, and skin of up to two-thirds of human beings without causing any apparent disease. S. aureus is also one of the most common pathogens isolated from clinical specimens causing skin and respiratory infections and invasive, distant site, and systemic infections, such as osteomyelitis, endocarditis, and sepsis. S. aureus can cause severe toxic shock through the release of pyrogenic exotoxins known as superantigens (SAgs), which trigger massive T-cell activation and a “cytokine storm” [1].

The interaction between S. aureus and its host involves recognition of the microbe by cells associated with innate immunity, such as macrophages (Mφs) and dendritic cells (DCs). These cells sense pathogens, upon phagocytosis [2], through pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs) that recognize invariant pathogen-associated molecular patterns (PAMPs) [3]. TLR2 and NOD1/2 proteins play a central role in this recognition: TLR2 recognizes peptidoglycan (PGN)–embedded lipoproteins and glycopolymers (wall teichoic acid and lipoteichoic acid [LTA]), whereas NOD1/2 recognize PGN structures, such as muramyl dipeptide (MDP) [4].

The innate response to TLR2 ligation is primarily proinflammatory [5]. TLR2 signalling upon heterodimerization with TLR1 or TLR6 recruits the myeloid differentiation factor 88 (MyD88), and the MyD88 adapter-like molecule (TIRAP/MAL) [4] followed by activation of the canonical nuclear factor (NF)–κB pathway and mitogen-activated protein kinases (MAPKs) (eg, ERKs, p38, and JNK). In addition, NOD
signalling in response to PGN fragments triggers RIP2-dependent activation of the canonical NF-κB pathway [6]. Together, this results in the production of proinflammatory chemokines and cytokines.

Scattered evidence suggests that TLR2 signalling can also elicit anti-inflammatory responses [1, 7]. TLR2 ligands can promote development of regulatory T cells (Tregs) and inhibit autoreactivity [8, 9] in an IL-10– and retinoic acid–dependent manner [10], can bias immune responses towards a Th2 profile [11], and can induce immune tolerance [12]. The modulatory effects of TLR2 signalling are not limited to responses to anti-CD3 antibodies or autoantigens but are also observed in response to bacteria. For example, TLR2 ligands on the S. aureus cell wall downregulate the T-cell response to staphylococcal SAGs by triggering IL-10 production by monocytes [13], and staphylococcal LTA can inhibit the proinflammatory response triggered by TLR3 agonists [14]. Thus, TLR2 signalling has an inherent plasticity to trigger proinflammatory and anti-inflammatory responses.

The repercussions of such a TLR2 signalling plasticity on the development of adaptive immunity to S. aureus have not been addressed experimentally. To identify the variables that regulate this plasticity, we looked at the molecular and functional effects of S. aureus and its PGN on human monocytes and monocyte-derived MΦs and DCs. We found that, in contrast to the proinflammatory response, the modulatory IL-10 response to PGN is CD14- and CD36-independent and requires PI3K activation. Such a modulatory response is restricted to monocyte-derived MΦs, whereas monocyte-derived DCs mount a proinflammatory response. This polarization of APCs translates into different adaptive immunity to S. aureus. PGN on MΦs triggers predominantly an IL-10 and a weak Th17 response. In contrast, PGN on DCs sets off a robust Th1/Th17 response.

**MATERIALS AND METHODS**

**Cells**

Human peripheral blood mononuclear cells (PBMCs) were isolated from healthy volunteers by Ficoll-Hystopaque density gradient centrifugation. Informed consent in compliance with the Office of Research Ethics at the University of Western Ontario was obtained from all individuals. Monocytes were purified from PBMCs by negative selection (Miltenyi Biotech) (purity >95%). Peripheral blood T cells were isolated with pan-T cell MACS columns (purity above 95%). Immature DCs were obtained by incubating 10⁶ monocytes in 2 mL of R-10 medium supplemented with recombinant human granulocyte macrophage colony-stimulating factor (50 ng mL⁻¹) and recombinant human IL-4 (50 ng mL⁻¹) for 7 days. One mL of medium was removed and 1.5mL of fresh medium with cytokines was replaced every 2–3 days. MΦs were obtained with macrophage-colony stimulating factor (50 ng mL⁻¹).

**Bacteria**

S. aureus strain Newman was used [15]. Bacteria were grown to stationary phase in tryptic soy broth, heat killed (80°C for 30 minutes), washed, and resuspended in sterile saline.

**Reagents**

Cells were cultured in Roswell Park Memorial Institute 1640 supplemented with 10% fetal calf serum. Monoclonal antibodies to CD14 and CD36 (from eBiosciences, R&D or Cell Sciences) and their appropriate isotype controls (eBiosciences) were used at 10 μg mL⁻¹. Inhibitors for PI3K (Wortmannin, LY294002), Src kinases (PP2), MEK1/2 (PD98059), and PKC (Rottlerin) (Sigma Aldrich or Calbiochem) were used at concentrations of up to 10 μM. TLR2 ligands (Saccharomyces cerevisiae zymosan, Pam3Csk4, Pam2CSK4, FSL-1, staphylococcal LTA, and PGN-Sandi ultrapure), as well as NOD2 ligand MDP were obtained from InvivoGen. The PGN-Sandi ultrapure was purified by detergent lysis and hydrolysis under basic conditions to eliminate lipophilic constituents. S. aureus PGN and Escherichia coli lipopolysaccharide (LPS) were purchased from Sigma-Aldrich. Contamination of PGN preparations by SAGs [16] was assessed by including a control group for T-cell activation by PGN alone. LPS contamination in stock solutions was ruled out by Limulus test and by direct measurement of endotoxin levels using the LAL Chromogenic Endpoint assay (Hycult Biotech).

**Cytokine Production**

PBMCs, monocytes, MΦs, and DCs were seeded in 96-well plates in R-10 medium at 10⁵ cells/well and stimulated for 18–24 hours with TLR ligands. To test the effect of inhibitors and antibodies, cells were pretreated for 1 hour prior to addition of TLR ligands. PBMCs or T cells and APCs were stimulated with heat-killed S. aureus or staphylococcal enterotoxin E (SEE) for 18–24 hours. Culture supernatants were assayed by enzyme-linked immunosorbent assay (BD Biosciences, eBiosciences, or R&D).

**Biochemistry**

Cells (5 × 10⁵ cells/group) were rested for 5 minutes at 37°C and then stimulated with TLR ligands for the indicated times. Lysates were prepared as previously reported [17].

**Statistical Analysis**

Statistical significance of differences was determined by analysis of variance with post hoc Bonferroni test using the Graphpad Instat software (Graphpad Instat Software). P < .05 was considered to be statistically significant.

**RESULTS**

**The Response of Human Monocytes to Staphylococcal PGN Includes Proinflammatory Cytokines and IL-10**

The staphylococcal PGN layer contains lipopeptides and glycolipids that can engage TLR2 on APCs and induce an IL-10
response by these cells [13]. To dissect the mechanism behind the modulatory IL-10 response of human monocytes to staphylococcal PGN, we examined the effect of different TLR2 ligands. We included MDP, a structure within PGN that binds NOD2, as a control for the involvement of this intracellular PRR. We found that selective TLR2/1 (Pam3Csk4), TLR2/6 (Pam2Csk4, FSL-1), and NOD2 (MDP) ligands induced a significant \( (P < .001) \) IL-6 response in human monocytes (Figure 1). However, other than a low IL-12p40 response induced by Pam3Csk4, these selective ligands did not induce significant production of IL-1\( \beta \), tumor necrosis factor (TNF)-\( \alpha \), IL-12p40, or IL-23. The lack of IL-1\( \beta \) production in response to TLR2/1 and TLR2/6 ligands is consistent with previous reports that suggest that an additional signal is needed to activate the inflammasome [18]. We did not detect IL-12p70 under these conditions of stimulation, which suggests that most of the IL-12p40 is released as IL-12p40 homodimers or as part of the IL-23 complex and not as functional IL-12.

In contrast to the synthetic TLR2/1 and TLR2/6 ligands, staphylococcal PGN and LTA and zymosan (a yeast-derived TLR2/6 and dectin-1 ligand) induced a much more diverse cytokine response that included proinflammatory cytokines (IL-1\( \beta \), TNF-\( \alpha \), IL-12p40, and low IL-23) and IL-10 (Figure 1). These responses were seen with PGN from different strains of \textit{S. aureus} (data not shown) and were mostly mediated by TLR2 signaling, as indicated by the minimal effect of the NOD2 ligand MDP and by the observation that an ultra-pure PGN, which lacks glycopolymers and lipopeptides and can only signal through NOD2 [19], did not induce IL-10 and induced much lower levels of proinflammatory cytokines than did standard PGN preparations (Figure 1; online only). The response profile of unfractionated PBMCs to TLR2 ligands and MDP was similar to, although quantitatively stronger than, that of monocytes (data not shown). Thus, for the next experiments, we used PBMCs because they are more representative of the in vivo scenario and recapitulate more closely the potential regulatory effects of T and B lymphocytes on the cytokine response during staphylococcal sepsis.

**Differential Requirement for CD14 in Proinflammatory and Modulatory Responses to Staphylococcal PGN**

To identify requirements for proinflammatory and modulatory responses to \textit{S. aureus}, we examined the contribution of CD14 and CD36, the two main TLR2 accessory molecules, using

![Figure 1](image_url)
TNF-\(\alpha\) and IL-10 productions as readouts for each type of response. CD14 blockade significantly reduced the TNF-\(\alpha\) response to PGN (\(P < .001\)) but did not have any significant effect on the IL-10 response (Figures 2a and b). The same result was obtained using monocytes (Figures 2c and d). Blockade of CD36 had no significant effect on IL-10 production and only a minimal effect on TNF-\(\alpha\) production at low concentrations of \(S.\) aureus PGN (Figures 2e and f). Based on these results, we concluded that the modulatory IL-10 response to \(S.\) aureus PGN is independent of CD14 and CD36, whereas the proinflammatory response requires CD14.

The Modulatory IL-10 Response to \(S.\) aureus PGN Requires PI3K Activation

The differential involvement of TLR2 accessory molecules prompted us to examine the signalling events associated with the modulatory response to staphylococcal PGN. We first used inhibitors for protein kinase C (PKC) (Rottlerin), Src kinases (PP2), MEK-1/-2 (PD98059), and PI3K (LY294002). Of these inhibitors, only LY294002 significantly inhibited the modulatory IL-10 response to PGN (\(P < .001\)) (data not shown). Inhibition of MAPKs had no effect, a conclusion corroborated by preserved ERK and p38 activation in the presence of reduced IL-10 response after PI3K inhibition.

Because LY294002 has been shown to have a nonspecific effect on cytokine production [20], we performed additional studies with another PI3K inhibitor, Wortmannin. We found that wortmannin effectively abolished activation of Akt, a downstream effect of PI3K activation (Figure 3a). PI3K inhibition significantly (\(P < .001\)) downregulated the IL-10 response to PGN (\(P < .001\)) (Figure 3b). Similar inhibition was also observed using isoform-selective PI3K inhibitors (data not shown).

In contrast to the downregulation of the modulatory IL-10 response, inhibition of PI3K significantly enhanced proinflammatory cytokine production by human PBMCs (Figure 3c–e). These effects were observed in different donors (Figure 3f). Thus, we concluded that activation of the PI3K/Akt pathway is required for PGN-induced IL-10 production and negatively regulates the proinflammatory response.

![Figure 2](image-url) The CD14 and CD36 accessory molecules are not required for the modulatory interleukin (IL–10) response to staphylococcal peptidoglycan (PGN). Human peripheral blood mononuclear cells (PBMCs) and monocytes were isolated and pretreated with medium or 10 \(\mu\)g mL\(^{-1}\) of anti-CD14 monoclonal antibody (mAb) (\(\alpha\)CD14), anti-CD36 mAb (\(\alpha\)CD36), or an isotype control (immunoglobulin [Ig] G), for 1 h prior to stimulation with PGN. After 24 h, IL-10 and tumor necrosis factor (TNF)-\(\alpha\) in the culture supernatant were measured by enzyme-linked immunosorbent assay. A, B, E, and F show responses of PBMCs, and C and D show responses for monocytes. Results represent mean \(\pm\) standard error of the mean of triplicate values. ***\(P < .001\).
Figure 3. PI3K activation is required for the modulatory interleukin (IL)–10 response and downregulates the proinflammatory response to staphylococcal peptidoglycan (PGN). A, time-dependent activation of Akt in human peripheral blood mononuclear cells (PBMCs) responding to PGN in the presence of Wortmannin (Wort) or the dimethyl sulfoxide (DMSO) vehicle control. B–E, PBMCs were preincubated with the PI3K inhibitor Wort (dashed line; 10 μM) or DMSO as vehicle control (solid line) for 1 h before PGN stimulation for 24 h. Next, cytokine accumulation (IL-10 [b], TNF-α [c], IL-12p40 [d], and IL-23 [e]) in the culture supernatants was measured by enzyme-linked immunosorbent assay. One representative experiment (mean ± standard error of the mean) of at least 4 experiments done in triplicate is shown. F, the profile of downregulation of modulatory IL-10 response and enhancement of proinflammatory response to Staphylococcus aureus PGN (10 μg mL⁻¹) by inhibition of PI3K with Wort (+) (10 μM) was observed in all donors tested. DMSO served as vehicle control (-). ns, not significant. *P < .05; **P < .01; ***P < .001.
The PGN-Induced PI3K-Dependent IL-10 Response Is Determined by the Type of APC

Because the sites colonized and infected by *S. aureus* have different representations of the 2 main types of professional APC (MΦs and DCs), we examined how differential involvement of TLR2 accessory molecules and signalling pathways played in these types of APC. We found that, on average, MΦs produced higher amounts of IL-10 than did DCs, required lower amounts of PGN to reach maximal response, and did not produce IL-12p40 (Figure 4a). In contrast, DCs produced large amounts of IL-12p40 (between 5 and 20 times higher than their IL-10 response). These data suggested that exposure to staphylococcal PGN differentially polarized APCs to a modulatory (in MΦs) versus proinflammatory (in DCs) phenotype.

The IL-10 response to PGN and to the TLR2-selective ligand LTA was inhibited by Wortmannin (Figure 4b). Interestingly, inhibition of PI3K increased *de novo* production of IL-12p40 in MΦs but not in DCs (Figure 4b). Therefore, PI3K is essential to maintain the modulatory phenotype and to downregulate the proinflammatory phenotype.

**Differential Imprinting of Adaptive T-cell Responses to *S. aureus* and Its SAgs by PGN-Polarized APC**

The cytokine polarization of MΦs and DCs as a result of differential signalling by staphylococcal PGN provided us with a unique opportunity to examine the regulation of adaptive T-cell responses to *S. aureus* by the innate immune response. We used heat-killed *S. aureus* to recapitulate more closely the effect of bacteria on the immune response and determined the profile of primary T-cell response to *S. aureus* under conditions in which the primary APCs were MΦs or DCs (Figure 5). Exposure to heat-killed *S. aureus* induced equal amounts of TNF-α production in both culture conditions, indicating a quantitatively similar exposure to the microbe. Interestingly, MΦs polarized the primary T-cell response to *S. aureus* to a predominant IL-10 and low IL-17 response with no Th1/interferon (IFN)—γ response. In contrast, DCs imprinted the primary T-cell response to high levels of Th1-inducing IL-12 (p40 and p70) and both Th1 and Th17 cytokines (IFN—γ and IL-17, respectively) with little IL-10 production. The same profiles of cytokine production were obtained when *S. aureus* cells were heat-killed at 80°C for 20 minutes or 30 minutes and independently of the bacteria being in log-phase or stationary-phase growth (data not shown). Altogether, we concluded that exposure to *S. aureus* triggers distinct profiles of adaptive T-cell responses depending on the type of primary APC responding to the bacteria.

To confirm the key role of the type of primary APC in determining modulatory versus proinflammatory immune responses to *S. aureus*, we tested the effect of exposure of MΦs and DCs to staphylococcal PGN on the T-cell response to staphylococcal SAgs toxins (Figure 6). Staphylococcal PGN downregulated the SEE-induced IL-2 response when monocytes or monocyte-derived MΦs were used as APCs, whereas it significantly enhanced the SEE-induced IL-2 response when DCs were the APCs. Thus, the innate response to PGN (modulatory or proinflammatory) differentially regulates adaptive T-cell responses to *S. aureus*, depending on the type of responding APC.

**Figure 4.** PI3K polarization of cytokine production induced by *Staphylococcus aureus* peptidoglycan (PGN) in monocyte-derived macrophages (MΦs) and monocyte-derived dendritic cells (DCs). *A*, monocyte-derived MΦs (*n* = 11 donors) or monocyte-derived DCs (*n* = 19 donors) were stimulated with *S. aureus* PGN (10 μg mL⁻¹) for 18 h and cytokine production (interleukin [IL]—10, IL-12p40) was measured by enzyme-linked immunosorbent assay. Points in the graph represent the mean of 1 experiment per donor done in triplicate. *B*, IL-10 and IL-12p40 responses of MΦs and DCs to PGN and lipoteichoic acid (LTA) upon preincubation with the PI3K inhibitor wortmannin (10μM) for 1 hour prior to 18 hour stimulation with PGN or LTA. Results represent mean ± SEM of triplicates. Wort: wortmannin; ***P < .001; ND: not detectable.
DISCUSSION

Ligation of TLRs by PAMPs is considered a trigger for proinflammatory cytokine responses. However, scattered evidence suggests that this view may be an oversimplification and that TLR signalling may have an inherent cell type-specific plasticity that translates into a more diverse range of responses [21]. Specifically for TLR2, viral but not bacterial ligands can induce type 1 IFN production by monocytes [22], and some ligands for this receptor can induce expression of IL-10 [23, 24] and downregulate rather than enhance a primary human T-cell response [13]. However, it is unknown what biological implications the modulatory response to TLR2 signalling may have in the context of microbial infections (ie, how they will imprint the ensuing adaptive response to the pathogen). Using S. aureus as a prototypic clinically important human pathogen, we show that differential TLR2 signalling leading to predominantly proinflammatory or modulatory responses is the result of selective involvement of the PI3K/Akt pathway in an APC-dependent manner (Mφs vs DCs) and differentially imprints the subsequent adaptive response as either an IL-10 response or a Th1/Th17 response.

The study of human immune responses to staphylococcal PGN preparations is useful to dissect the mechanism of immunity to S. aureus. The human being is a primary reservoir of this microbe, and mouse models show a relative resistance to S. aureus and its toxins, including SAgs. The cytokine response of human PBMCs to S. aureus PGN preparations is more diverse...
than the response seen with selective ligands of TLR2/1 or TLR2/6 complexes, as also seen in genomic studies of patients with staphylococcal sepsis [25]. This response has also been seen in a protein A–deficient S. aureus strain (data not shown), further supporting the idea that the cytokine response is induced by PGN-embedded TLR2 ligands, such as glycopolymers and lipopeptides. Furthermore, our data with ultrapure PGN corroborate that this response is primarily mediated by TLR2 signalling. The diverse cytokine response to staphylococcal PGN by human PBMCs illustrates that TLR2 signalling can induce not only a proinflammatory response but also a modulatory response, a conclusion consistent with emerging data showing that TLR2 deficiency is associated with an exacerbated inflammatory response to S. aureus [26].

Our data reveal that proinflammatory and modulatory TLR2-induced responses can be uncoupled, because they reflect not only the sheer magnitude of TLR2 engagement but also differences in signalling requirements. One of the differences is in the use of the TLR2 accessory molecule CD14. We confirmed that CD14 is critical for the TLR2 proinflammatory response by human monocytes [27]; neither CD14 or CD36 are required for the IL-10 response. The uncoupling of proinflammatory versus modulatory TLR2 signalling opens up the possibility of targeting CD14 to manipulate the balance between proinflammatory and modulatory responses induced by this receptor.

The PI3K/Akt pathway plays a modulatory role in early phases of TLR2 signalling, limiting Th1/Th17 polarization. As observed in mice [28], we found that this pathway negatively regulated the proinflammatory response of primary monocytes to staphylococcal PGN, which is an effect that may involve GSK3 [29]. Similar results have been reported by Michalek’s group using human PBMCs and LPS from Porphromonas gingivalis, an unusual LPS that binds TLR2 [30], and by Vogel’s group using ligation of FcγR and E. coli LPS in murine MΦs [31].

These findings are in marked contrast to those from other groups that reported that PI3K activation is required for proinflammatory TLR2 signaling [32, 33]. The basis for the discrepancy is unclear, but our data suggest that it could be attributable to the state of differentiation of the responding cells (MΦs vs DCs) determining differential PI3K activation.

Whether the predominant PI3K activation and IL-10 response upon TLR2 signalling in monocytes and MΦs reflect preferential TLR2/6 signaling or TLR2/1 signaling is unclear [13, 34, 35]. TLR1, TLR2, and TLR6 have PI3K binding motifs, but these receptors lack intrinsic kinase activity and thus require another kinase for phosphorylation. An attractive possibility to explain differential recruitment and activation of PI3K is to claim the selective involvement of an adapter protein, such as MAL [33, 36], or of regulatory enzymes, such as Lyn or SHP-1 [37, 38], under some conditions of TLR2 ligation. Preliminary evidence indicates that MAL expression is not significantly different between primary human MΦs and DCs (data not shown), and it is unlikely that differences in MAL expression explain the predominant PI3K activation and IL-10 response in monocytes and MΦs.

The predominant IL-10 response of monocyte-derived MΦs to staphylococcal PGN is reminiscent of the alternative activation of MΦs that occurs upon exposure to IL-4 and IL-13 in the context of strong Th2 immune responses to protozoan and helminth infections. Alternative activation of MΦs has also been observed in some Th1-prone environments, such as infections by Mycobacterium tuberculosis [39] or Francisella tularensis [40], and infections due to respiratory syncytial virus [41]. This alternative activation may minimize tissue injury, but may also become an escape mechanism for the pathogen [42]. Of interest, TLR2 signalling is also required for alternative activation in some of these infections [40]. However, it is unlikely that the modulatory TLR2 signalling in response to S. aureus is the result...
of alternative activation of MΦs. First, there is no source of IL-4 and IL-13 in our system to generate MΦs. Second, alternative activation of MΦs correlates with inhibition of the PI3K/Akt pathway [42], contrary to the requirement for activation of this pathway in the modulatory response to *S. aureus* PGN. Third, induction of alternative activation of MΦs by IL-4 and IL-13 antagonizes the secretion of proinflammatory cytokines, such as IL-6 or TNF-α [43], whereas, in our system, the secretion of these cytokines was not affected. Thus, the modulatory TLR2 signalling in response to *S. aureus* is unlikely to be the result of alternative activation of MΦ but, rather, reflects an inherent plasticity of TLR2 signalling in different APCs.

The balance between proinflammatory and regulatory TLR2 signalling is determined by the APC type initially responding to *S. aureus*. Such a balance translates into a primarily IL-10 response, capable of reducing T-cell responses, or into a Th1/Th17 response, which enhances proinflammatory responses. Our findings provide an explanation for different outcomes of *S. aureus* encounters with humans, ranging from harmless commensalism to toxic shock and death. *S. aureus* colonization, primarily occurring in the upper respiratory tract, will be facilitated by the predominant presence of MΦs in the submucosa. These cells would favor a low grade of inflammation and minimize the chances of toxic shock syndrome due to staphylococcal SAGs. In contrast, the presence of *S. aureus* in skin may pose a threat upon cutaneous injury, triggering a proinflammatory response because of the predominance of DCs in this location. This paradigm complements the observations that IL-17 is required for proper immunity to *S. aureus* infections [44] and that defects leading to abnormal IL-17 function (eg, STAT-3 required for proper immunity to *S. aureus* infections) increases susceptibility to *S. aureus* infection [45].

Differential signalling by TLR2 ligands has implications for the development of adjuvants, particularly for mucosal immunization. Our results stress that not only the TLR2 ligand but also the type of responding APC are key determinants of adjuvanticity. An adjuvant needs to maximize proinflammatory and minimize modulatory TLR2 signalling. Thus, identifying structural requirements that differentiate one from the other and modifying the TLR2 ligand accordingly may help to optimize adjuvanticity. A potential target may be CD14, which is needed for proinflammatory signaling but is not needed for modulatory signaling.

In summary, the modulatory role of TLR2 in *S. aureus* infections results from an inherent signaling plasticity of this receptor on different types of APCs. Differential staphylococcal PGN-induced TLR2 signalling to APCs imprints the adaptive response to *S. aureus* to either a IL-10 response orchestrated by MΦs or a predominantly proinflammatory Th1/Th17 response directed by DCs. This framework may explain the wide range of *S. aureus*-host interactions, from commensalism to pathogenicity.

### Supplementary Data

Supplementary figure is available at *The Journal of Infectious Diseases* online.

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