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Selective Regulation of IL-10 Signaling and Function by Zymosan

Zhimei Du,* Erin Kelly,* Ingrid Mecklenbräuker,† Lucila Agle,‡ Carmen Herrero,‡ Paul Paik,‡ and Lionel B. Ivashkiv1*‡

Balanced activity of pro- and anti-inflammatory cytokines during innate immune responses is required to allow effective host defense while avoiding tissue damage and autoimmunity. Induction of cytokine production after recognition of pathogen-associated molecular patterns (PAMPs) by innate immune cells has been well demonstrated, but modulation of cytokine function by PAMPs is not well understood. In this study we show that stimulation of macrophages with zymosan, which contains PAMPs derived from yeast, rapidly extinguished macrophage responses to IL-10, a suppressive cytokine that limits inflammatory tissue damage but also compromises host defense. The mechanism of inhibition involved protein kinase Cβ and internalization of IL-10R, and was independent of TLR2 and phagocytosis. Inhibition of IL-10 signaling and function required direct contact with zymosan, and cells in an inflammatory environment that had not contacted zymosan remained responsive to the paracrine activity of zymosan-induced IL-10. These results reveal a mechanism that regulates IL-10 function such that antimicrobial functions of infected macrophages are not suppressed, but the activation of surrounding noninfected cells and subsequent tissue damage are limited. The fate of individual cells in an inflammatory microenvironment is thus specified by dynamic interactions among host cells, microbes, and cytokines that determine the balance between protection and pathology. The Journal of Immunology, 2006, 176: 4785–4792.

Macrophages are innate immune cells that recognize, phagocytose, and kill microbial pathogens. Interactions between macrophage pattern recognition receptors, such as TLRs, and pathogen-associated molecular patterns (PAMPs) result in macrophage activation and secretion of both pro- and anti-inflammatory cytokines. Proinflammatory cytokines such as TNF-α are important for host defense, whereas anti-inflammatory factors such as IL-10 are produced as part of a feedback inhibition loop that limits inflammation and thereby limits tissue damage and the emergence of autoimmunity. However, IL-10 can also impair microbial clearance and facilitates the local outgrowth of microorganisms (1). Therefore, an important issue is how the expression and function of IL-10 are regulated to achieve a balance that ensures effective immunity but limits tissue damage.

Historically, the balance between pro- and anti-inflammatory cytokines has been considered to be determined primarily by regulation of the expression of cytokines and their receptors (2). IL-10 is a potent anti-inflammatory cytokine that is very effective at suppressing TNF-α production in response to individual macrophage activators, such as the TLR4 ligand LPS. However, emerging evidence suggests that in more complex inflammatory settings IL-10, even when expressed at high levels, may not be effective in suppressing TNF-α production. For example, TNF-α and IL-10 are coexpressed during inflammation (3–8) and, following infection, the production of IL-10 appears to correlate both with the severity of the inflammatory insult and the plasma concentration of TNF-α (6, 8). Moreover, human trials of IL-10 therapy have shown lack of efficacy in suppressing TNF-α-dependent inflammation (9, 10).

These observations suggest that there are mechanisms that compromise the anti-inflammatory function of IL-10 during inflammation. To explore this phenomenon, we used zymosan stimulation of macrophages as a model system to study the effects of PAMPs on the function of IL-10.

Zymosan is a cell wall preparation of Saccharomyces cerevisiae that has been used for over 50 years as a model phagocytic and inflammatory stimulus both in vivo and in vitro (11–13). Zymosan is composed of β-glucans, mannans, chitin, and activates several macrophage receptors, including TLR2, dectin-1, the mannose receptor, and CD11b/CD18 (complement receptor 3). Recent reports have advanced the notion that simultaneous engagement of different macrophage receptors, such as TLR2 and dectin-1, by zymosan synergistically activates inflammatory pathways leading to increased TNF-α production and an oxidative burst (11, 14). Dectin-1, a C type lectin that recognizes β-glucans, also signals independently of TLR2 by activating Syk kinase via an ITAM, leading to induction of IL-2 and IL-10 production (15). An important role for zymosan and dectin-1 in inducing chronic autoimmune arthritis via activation of innate immune mechanisms and cytokine production has been demonstrated (16). In this study, we investigated whether zymosan regulates cytokine function by modulating signal transduction. We found that although zymosan induces both TNF-α and IL-10, the function of IL-10 is blocked at the signaling level by a protein kinase C (PKC)-dependent mechanism that requires direct contact of cells with zymosan.

Materials and Methods

Cell culture and reagents

Primary human macrophages and murine thioglycollate-elicited peritoneal macrophages were obtained as previously described (17, 18). IL-10 and IFN-γ were purchased from PeproTech, zymosan from Molecular Probes, and Pam3CysSer(Lys)4 from EMC Microcollections. Abs against Stat1,
Stat3, Jak1, and Tyk2 were purchased from Cell Signaling Technology or BD Transduction Laboratories. GF109203X, SB203580, PD98059, actinomycin D, PKC\textsubscript{β}-specific C2-4 inhibitory peptide (SLNPEWNET), and PKC\textsubscript{ε}-specific inhibitory peptide (EAVSLKPT) were purchased from Calbiochem. Cytochalasin D was purchased from Sigma-Aldrich.

**Immunoblotting, EMSA, and immunoprecipitation**

Whole cell extract preparation, immunoblotting, immunoprecipitation, and EMSA were performed as previously described (17).

**Immunofluorescence microscopy**

Cells were cultured in LabTek chamber slides (Nalge Nunc International) and processed for immunofluorescence studies. Briefly, cells were fixed and permeabilized with cold methanol, and stained with primary rabbit anti-Stat3 or rabbit IgG as control, and Alexa Fluor 488-conjugated secondary Abs (Molecular Probes). Cells were imaged using a Leica DC 200 digital camera (Leica, Switzerland) attached to a Zeiss Axioplan microscope. Images were imported into Adobe Photoshop 7.0.

**Cell surface biotinylation**

The assay was performed as described (19). Cells were washed three times in PBS at 4°C and labeled with 0.5 mg/ml sulfo-NHS-biotin (N-hydroxy-sulfosuccinimide biotin; Pierce) in PBS for 30 min on ice. Lysis buffer was added for 30 min on ice and clarified lysates were then precipitated with avidin-agarose beads.

**ELISA and flow cytometry**

For ELISA, paired TNF-α, IL-10, and IL-6 capture and detection Abs were purchased from R&D Systems and used in a sandwich ELISA, according to the instructions of the manufacturer.

**Gene expression analysis**

The 1 μg of total RNA was reverse transcribed using oligo(dT) primers and Moloney murine leukemia virus reverse transcriptase. Real-time PCR was performed as previously described (18). mRNA amounts were normalized relative to GAPDH mRNA.

FACS analysis of IL-10R levels at the cell surface was conducted using the FLUOROKINE kit according to the instructions of the manufacturer (R&D Systems). A total of 10\textsuperscript{5} human primary macrophages were washed twice with 1× RDF1 (R&D flow specific-1) wash buffer, resuspended in 25 μl of PBS, and incubated with 30 ng of biotinylated IL-10 at 4°C for 1 h. As a negative staining control, an identical sample of cells was stained with 50 ng of a biotinylated negative control protein (soybean trypsin inhibitor) provided by the manufacturer. As a specificity control, anti-human IL-10 Ab was used to block the interaction of biotinylated IL-10 with its receptor. Cells were then washed three times in RDF1 buffer, and incubated with avidin-conjugated FITC (2.2 μg/ml) at 4°C for 30 min. The cells were then washed twice and analyzed with a FACScan cytometer and CellQuest software (BD Biosciences). Cells present in the macrophage gate as defined by forward and side light scatter were analyzed, and dead cells were excluded by propidium iodide staining.

For phagocytosis experiments, human primary macrophages were preincubated with or without 4 μM cytochalasin D for 1 h followed by stimulation with Texas Red-labeled zymosan (Molecular Probes) for 1 h at 37°C. A weak but specific signal was obtained by exciting the Texas Red fluorochrome by a standard argon laser emitting at 488 nm and detection by the 650 nm FL2 filters on a FACScan cytometer and data were analyzed using CellQuest software (BD Biosciences).

**FIGURE 1.** Zymosan abrogates feedback inhibition mediated by endogenous IL-10. **A**, Human primary macrophages were stimulated with zymosan (ratio of particles to cells was 9:1) and with Pam\textsubscript{3}CysSer(Lys)\textsubscript{4} (100 ng/ml) for the indicated times. Supernatants were collected and cytokine levels were measured using ELISA. One representative experiment of three is shown. **B**, Cells were lysed and Stat3 activation was measured using immunoblotting. **C**, Human primary macrophages were stimulated with zymosan and Pam\textsubscript{3}CysSer(Lys)\textsubscript{4} for 6 h with (+) or without (−) pre-treatment with blocking Ab against IL-10 or control Ab for 5 min. **D**, A Transwell experiment was performed by plating human macrophages in both top and bottom chambers that are separated by a permeable membrane with a pore size of 0.8 μm. Zymosan was added to the bottom chamber for the indicated times.
Transfection of inhibitory peptides

Peptides were transfected into primary human macrophages using the Amaxa Nucleofector apparatus according to the manufacturer’s specifications. Briefly, peptides (50 μM) were incubated with 10^7 human primary macrophages in 100 μl of Human Monocyte Nucleofector Solution provided in the Amaxa Human Monocyte Nucleofector kit and electroporated using nucleofector program Y-01. Cells were then transferred into 0.5-ml Human Monocyte Nucleofector medium (Amaxa) and zymosan and IL-10 were added 1 h later.

Results

Modulation of cytokine production and function by zymosan

IL-10 is induced by PAMPs and can mediate a feedback inhibition loop that limits inflammatory cytokine production. The suppressive effects of IL-10 on TLR-induced cytokine production are well documented (1). We compared the regulation of TNF-α and IL-10 production and IL-10 function by the purified TLR2 ligand Pam3CysSer(Lys)4 and by zymosan, which activates TNF-α production via TLR2 but also engages additional pattern recognition receptors. Primary human blood-derived macrophages were stimulated with zymosan or with the soluble TLR2 ligand Pam3CysSer(Lys)4 at concentrations that activated similar levels of TLR2-induced signal transduction (data not shown), and cytokine production was measured. Zymosan induced higher levels of TNF-α and IL-10 than did Pam3CysSer(Lys)4 (Fig. 1A). Next, we examined whether the IL-10 expressed in these cultures signaled in an autocrine manner. IL-10 activates the tyrosine phosphorylation and DNA binding activity of Stat3, which is required for its anti-inflammatory activity (20, 21). Stat3 activation was consistently observed 6 h after stimulation with Pam3CysSer(Lys)4 (Fig. 1B, lanes 6–8), and the kinetics of Stat3 activation were consistent with the kinetics of IL-10 production (Fig. 1A). Endogenous IL-10 was mainly responsible for Stat3 activation in Pam3CysSer(Lys)4-stimulated macrophages, as Stat3 activation was almost completely blocked by anti-IL-10 but not by control Abs (Fig. 1C). In marked contrast to Pam3CysSer(Lys)4-stimulated macrophages, Stat3 activation was completely absent in zymosan-stimulated macrophages (Fig. 1B, lanes 2–4). This result suggested that either the IL-10 present in culture supernatants of zymosan-stimulated macrophages was not biologically active, or that the macrophages had become refractory to IL-10.

We then investigated the signaling capacity of zymosan-induced IL-10 by using Transwell cultures. Cells that interacted with zymosan were separated from zymosan-free cells by a permeable membrane. As shown in Fig. 1D, IL-10, which was induced in response to zymosan (bottom chamber), was active, as it activated Stat3 in macrophages in the upper wells of Transwell plates that were not directly exposed to zymosan. These data suggest that zymosan-induced IL-10 production, but inhibited IL-10 autocrine function by suppressing IL-10-induced signal transduction. Importantly, this inhibitory effect required direct zymosan-cell interaction. The loss of IL-10 function interrupts IL-10-mediated feedback inhibition of cytokine production and explains the higher levels of TNF-α produced by macrophages stimulated with zymosan (Fig. 1A).

Inhibition of IL-10 signaling

To investigate the mechanism of inhibition of IL-10 signaling by zymosan, we first tested the effects of zymosan on signaling by exogenous IL-10 that was added to macrophage cultures. As expected, stimulation of macrophages with IL-10 induced Stat3 tyrosine phosphorylation, nuclear localization, and DNA binding activity, whereas preincubation with zymosan effectively blocked all these effects (Fig. 2, A–D). Inhibition of IL-10 signaling by zy-
mosan was concentration-dependent (Fig. 2B). Inhibition of IL-10 signaling was not observed even with saturating concentrations of the TLR2 ligand Pam3CysSer(Lys)12 (Fig. 2A and data not shown), indicating that activation of TLR2 is not sufficient to suppress IL-10 signaling. Lack of suppression of IL-10 signaling by TLR2 is consistent with the established ability of IL-10 to suppress TLR function (1). A kinetic experiment showed that inhibition of IL-10 signaling by zymosan was time-dependent. Addition of zymosan at the same time, or 5 min before addition of IL-10 had minimal effect on IL-10 signaling (Fig. 2C). Suppression of IL-10 signaling by zymosan was readily apparent after 15 min of pretreatment with zymosan, and sustained even after prolonged exposure of macrophages to zymosan for 16 h (Fig. 2C); comparable viability of macrophages with or without zymosan was verified using cell counts and trypan blue exclusion (data not shown). IL-10 signaling was blocked at every time point when the incubation with IL-10 was varied from 5 to 90 min (data not shown), indicating that zymosan did not alter the kinetics of IL-10-induced Stat3 activation, but inhibited activation. The strong and long lasting effect of zymosan on IL-10 signaling suggested that zymosan would have significant functional consequences in terms of inhibiting IL-10 induction of gene expression. Indeed, zymosan inhibited the expression of IL-10-inducible genes concomitantly with suppression of signaling (Fig. 2E). These results demonstrate that zymosan suppressed IL-10 function concomitantly with suppression of IL-10 signaling.

In contrast to IL-10, IFN-γ is a strong activator of macrophages that also activates Jak-STAT signaling. We investigated the specificity of zymosan-induced inhibition of signaling by determining the effects of zymosan on IFN-γ-induced signaling and gene expression. Zymosan had minimal effect on activation of Stat1 by IFN-γ (Fig. 2F) and did not suppress activation of gene expression by IFN-γ (Fig. 2G). Thus, zymosan preferentially suppressed signaling by the deactivating cytokine IL-10.

Inhibition of IL-10 signaling by zymosan is a direct effect

Zymosan induces many proinflammatory cytokines, mediators, and reactive oxygen intermediates that could potentially suppress IL-10 signaling (22). We addressed a potential role for zymosan-induced soluble mediators in inhibition of IL-10 signaling by using Transwell cultures (Fig. 3A). Zymosan was added to only the top chamber for 30 min and IL-10 or IL-6 was then added to both chambers. IL-6 signaling was inhibited in macrophages in both top and bottom chambers, consistent with the previously reported inhibition of IL-6 signaling by inflammatory factors such as IL-1 and reactive oxygen intermediates (23). In contrast, IL-10 signaling was inhibited in cells in the top chamber that directly contacted zymosan, but not cells in the bottom chamber that were exposed only to zymosan-induced secreted factors. In addition, IL-10 signaling was not inhibited by IL-1 or TNF-α (Fig. 3B), inflammatory factors that are induced by zymosan and strongly inhibit IL-6 signaling (23, 24). These results indicate that zymosan-induced inhibition of IL-10 signaling is not mediated by soluble factors and suggest that zymosan may suppress IL-10 signaling by a direct pathway.

A major mechanism of inhibition of Jak-STAT signaling is de novo induction of expression of suppressors of cytokine signaling (SOCS) proteins. However, data from Fig. 2C suggested that SOCS proteins might not play a major role here because zymosan inhibited IL-10 signaling rapidly (within 15 min), whereas inhibition mediated by SOCS is typically apparent only after several hours. To obtain experimental support for the notion that inhibition of IL-10 signaling was not mediated by SOCS or other inducible factors in our system, we used actinomycin D and cycloheximide to block mRNA and protein synthesis, respectively. Preincubation with both inhibitors had no effect on zymosan-mediated inhibition of IL-10 signaling (Fig. 3C). Thus, inhibition of IL-10 signaling by zymosan did not require de novo synthesis of inhibitory proteins and likely was independent of SOCS proteins. Taken together, the data indicate that zymosan suppressed IL-10 signaling by a direct pathway, independent of the production of inhibitory molecules.

**Zymosan inhibits of IL-10 signaling through a PKC-dependent pathway**

We wished to identify the zymosan-induced signaling pathway that inhibited IL-10 signaling. Our group and others have described rapid inhibition of Jak-STAT signaling that is dependent upon PKC, ERK, or p38 kinases and is mediated by posttranslational modification of signaling molecules (17, 23–31). We used specific kinase inhibitors to investigate the role of these kinases in zymosan-induced inhibition of IL-10 signaling. As shown in Fig. 4A, inhibitors of ERKs and p38 had no effect on IL-10 signaling. In contrast, the PKC inhibitor GF109203X reversed zymosan-mediated inhibition of IL-10 signaling (Fig. 4B). These results demonstrate that zymosan induced inhibition of IL-10 signaling through a PKC-dependent pathway.
Because of potential differences between murine elicited peritoneal macrophages and human monocyte-derived macrophages, we investigated the role of PKCβ in suppression of IL-10 signaling by zymosan in human macrophages. Several approaches to RNA interference-mediated suppression of PKCβ expression in primary human macrophages were not successful at the protein level, and instead we used a specific peptide inhibitor of PKCβ that blocks PKCβ translocation to membranes and thus inhibits its interaction with substrates. Inhibition of PKCβ by a specific peptide inhibitor abrogated the ability of zymosan to inhibit IL-10 signaling in human macrophages, whereas a peptide inhibitor of PKCe had no effect (Fig. 4C). These results, taken together with the results obtained using murine PKCβ-deficient macrophages (Fig. 4B), support a role for PKCβ in the regulation of IL-10 responses by zymosan.

**Phagocytosis, opsonization, TLR2, and CD11b are dispensable for zymosan-mediated inhibition of IL-10 signaling**

Zymosan is rapidly phagocytosed and directly activates several macrophage receptors that can potentially activate PKCβ and thereby inhibit IL-10 signaling, including TLR2, CD11b/CD18 (CR3), dectin-1, and the mannose receptor. In addition, zymosan can be opsonized by serum proteins such as Abs and complement, thus activating Fc and complement receptors that also activate PKCβ and might inhibit IL-10 signaling. We wished to address the role of phagocytosis, opsonization, and known zymosan receptors in mediating inhibition of IL-10 signaling. Latex beads of a size similar to zymosan that were phagocytosed to a comparable extent as zymosan, and larger Ab-coated erythrocytes that were effectively phagocytosed, had no effect on IL-10-induced activation of Stat3 (18) (Fig. 5A). Thus, phagocytosis alone was not sufficient to inhibit IL-10 signaling. To address whether phagocytosis was necessary for inhibition of IL-10 signaling, we used cytochalasin D to block phagocytosis. As shown in Fig. 5B, cytochalasin D effectively blocked phagocytosis of zymosan, but had no effect on zymosan-mediated inhibition on IL-10 signaling. Inhibition of IL-10 signaling by zymosan also did not require opsonization by serum proteins (such as complement or Abs), as IL-10 signaling was effectively inhibited under serum-free conditions (Fig. 5C).

Both zymosan and Pam3CysSer(Lys)4 activate TLR2 (34–36). Zymosan inhibited IL-10 signaling but Pam3CysSer(Lys)4 did not (Fig. 2), showing that TLR2 ligation was not sufficient for inhibition of IL-10 signaling. However, it was still possible that TLR2 was necessary for inhibition to occur, secondary to cooperative interactions with other macrophage receptors. The possibility that TLR2 was required for inhibition of IL-10 signaling was addressed using thioglycolate-elicited peritoneal macrophages from TLR2−/− mice. Zymosan inhibited IL-10 signaling in macrophages from genetically matched control and TLR2−/− mice (Fig. 5D), supressing zymosan-mediated inhibition on IL-10 signaling. However, it was still possible that TLR2 was necessary for inhibition to occur, secondary to cooperative interactions with other macrophage receptors. The possibility that TLR2 was required for inhibition of IL-10 signaling was addressed using thioglycolate-elicited peritoneal macrophages from TLR2−/− mice. Zymosan inhibited IL-10 signaling in macrophages from genetically matched control and TLR2−/− mice (Fig. 5D), showing that TLR2 is dispensable in zymosan inhibitory function. CD11b was also not necessary for zymosan-induced inhibition of IL-10 signaling (Fig. 5E). The results, taken together, indicate that phagocytosis did not play an important role, and that serum proteins, Fc receptors, TLR2, and CD11b were dispensable for inhibition of IL-10 signaling.

**Down-regulation of cell surface IL-10 receptor levels by zymosan**

PKC has been shown to induce internalization of cell surface receptors mediated by phosphorylation-dependent activation of internalization motifs present in receptor cytoplasmic domains (37). We investigated the effects of zymosan on macrophage cell surface IL-10R levels. Stimulation with zymosan led to a rapid decrease of cell surface IL-10R binding sites, as assessed using biotinylated

**FIGURE 4.** Inhibition of IL-10 signaling by zymosan through a PKCβ-dependent pathway. A, Human primary macrophages were incubated with 4 μM GF109203X, 10 μM SB203580, and 50 μM PD980591 for 30 min to inhibit PKC, p38 kinase, and MEK/ERKs, respectively. Zymosan was then added 1 h before the cells were stimulated with IL-10. B, Thioglycolate-elicited peritoneal macrophages from wild-type, PKCβ−/−, PKCε−/− mice were stimulated with IL-10 with or without pretreatment of zymosan for 1 h. C, Human primary macrophages were transfected with the indicated inhibitory peptides followed by 1 h of zymosan treatment before stimulation of cells with IL-10. The C2-4 PKCβ-specific inhibitory peptide (SLNP6WNET) is a nonapeptide derived from the RACK1 binding site in the C2 domain of PKCβ (218–226), which has been widely used to inhibit cellular function of PKCβ (42–44). PKCε-specific inhibitory peptide (V1–2: EAVSLKPT) is an octapeptide derived from the V1 region of PKCε that selectively inhibits the translocation and function of PKCε to subcellular sites (44, 45).
IL-10 that primarily binds to IL-10R1 (Fig. 6A). Cell surface expression of IL-10R2, a signaling component of the IL-10R that plays a less important role in ligand binding, was nearly completely absent after zymosan treatment, as assessed by immunoblotting of precipitated plasma membrane proteins (Fig. 6B). In contrast, total cellular IL-10R2 levels did not change (Fig. 6B). These results suggest

FIGURE 6. Inhibition occurs at a proximal step in IL-10 signal transduction. A, After treatment with zymosan for 20 min, cell surface IL-10R expression of human primary macrophages was measured using biotinylated IL-10 and flow cytometry. B, Biotinylated human macrophage cell surface proteins were precipitated with avidin-agarose beads and analyzed by immunoblotting. C, Jak1 and Tyk2 immunoprecipitates were analyzed by immunoblotting. D, Scha- metric model is shown of dynamic interactions of host cells, microbes, and cytokines.
that zymosan induces internalization of the IL-10R, and are consistent with previous work demonstrating rapid cycling of IL-10R between the cell surface and intracellular compartments (22). Decreased cell surface expression of IL-10R would suppress proximal steps in IL-10 signal transduction. Indeed, activation of IL-10R-associated Jak1 and Tyk2 was suppressed after treatment with zymosan (Fig. 6C). These results demonstrate that zymosan inhibits IL-10 signaling at a proximal step, upstream of STATs.

Discussion

Appropriate balance between pro- and anti-inflammatory cytokines is essential for a successful innate immune response that clears infectious pathogens but limits tissue damage and autoimmunity. The paradigm has been that this balance is achieved primarily by regulating the expression of cytokines and inflammatory mediators. Our recent work has extended this paradigm to take into account that regulation of cytokine activity contributes to cytokine balance in chronic inflammation (18, 38–41). In this study, we further extend this notion to inflammation in the context of an innate response to PAMPs. Not only is cytokine function regulated at sites of inflammation, but regulation in an inflammatory microenvironment differs depending upon whether cells directly contact microbes (Fig. 6D). The outcome is that the action of homoeostatic cytokines such as IL-10 is specifically regulated such that IL-10 can exert homoeostatic functions without a concurrent compromise in host defense against intracellular microbes. This kind of mod-ulation is not limited to zymosan, as similar effects were also observed with the pathogenic bacterium Staphylococcus aureus (Z. Du, unpublished observation).

TLRs are a critical link in recognition of microorganisms and the initiation of innate host defense, and are also potent inducers of both TNF-α and IL-10. However, we have demonstrated in this study that TLR2, the major TLR activated by zymosan, is not essential for zymosan-mediated inhibition on IL-10 signaling. Thus, signaling pathways different from the MyD88/TIRAP-de-pendent NF-κB and MAPK pathways downstream of TLR2 are involved in inhibition of IL-10 (Fig. 6D). In addition, IL-10 sig-naling was not inhibited by the TLR4 activator LPS (Z. Du, un-published observation), therefore MyD88-independent pathways downstream of TLRs are not sufficient to inhibit IL-10 signaling. It follows that ligation of macrophage receptors other than TLRs by zymosan is required for inhibition of IL-10 signaling.

Of several zymosan receptors that are likely ligated in our system, we have ruled out a role for Fc receptors and complement receptors, including CD11b/CD18. The identity of the zymosan receptor that mediates inhibition of IL-10 signaling remains unknown, but such a receptor must be capable of activating PKCβ. One intriguing possibility is the β-glucan receptor dectin-1. Sim-ilar to other receptors previously shown to inhibit Jak-STAT sig-naling that activates kinases of the Zap70/Syk family (18, 29, 30), dectin-1 activates Syk, but whether dectin-1 activates PKCβ is not known. Our attempts to date to block dectin-1 have been only partially successful, and the effects of blocking dectin-1 on IL-10 signaling have been modest and donor-dependent. Thus, the def-initive resolution as to whether dectin-1 suppresses IL-10 signal-ing awaits the generation of dectin-1-deficient mice. It is possible that several zymosan receptors that activate Syk and PKCβ work synergistically in the inhibition of IL-10 signaling, and the contribu-tion of any individual receptor will be minor.

Taken together, our data indicate that the interplay between mi-crobes, host cells, and cytokines plays an important role in the early innate immune response. The combination of rapid expres-sion of IL-10 and regulation of IL-10 signaling may permit a rapid induction of a homoeostatic response to inflammation while at the same time fine tuning this response to avoid excessive immuno-suppression. Also, the preferential inhibition of IL-10 on zymosan-interacting cells, but not on zymosan-free cells, could in part ex-plan how individual cell fate is determined at local sites of infection and may provide insight into the therapeutic failure of IL-10 in autoimmune/inflammatory diseases. The model we pro-pose (Fig. 6D) with multiple functional interactions among zymo-san, cells, and cytokines is that multiple, sequential interactions contribute to cell fate determination. We presume that this kind of complex regulation of cytokine signaling exists in part to permit a broad range of regulatory capabilities.

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Disclosures

The authors have no financial conflict of interest.

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