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Cutting Edge: All-trans Retinoic Acid Down-Regulates TLR2 Expression and Function

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A major consequence of microbial infection is the tissue injury that results from the host inflammatory response. In acne, inflammation is due in part to the ability of Propionibacterium acnes to activate TLR2. Because all-trans retinoic acid (ATRA) decreases inflammation in acne, we Investigated whether it regulates TLR2 expression and function. Treatment of primary human monocytes with ATRA led to the down-regulation of TLR2 as well as its coreceptor CD14, but not TLR1 or TLR4. The ability of a TLR2/1 ligand to trigger monocyte cytokine release was inhibited by pre- and cotreatment with ATRA; however, TLR4 activation was affected by cotreatment only. ATRA also down-regulated monocyte cytokine induction by P. acnes. These data indicate that ATRA exerts an anti-inflammatory effect on monocytes via two pathways, one specifically affecting TLR2/1 and CD14 expression and one independent of TLR expression. Agents that target TLR expression and function represent a novel strategy to treat inflammation in humans. The Journal of Immunology, 2005, 174: 2467–2470.

Retinoic acid (ATRA) regulates TLR expression and function. ATRA (Sigma-Aldrich) was diluted in DMSO to a stock concentration of 10^{-2} M and stored at −80°C. Further dilutions were made in amber tubes (USA Scientific) and aliquoted to prevent repeated freeze thaw cycles. All experiments were conducted with 10^{-7} M of ATRA because it was found to be toxic to monocytes at higher concentrations. The cell viability following ATRA treatment was comparable to the control cells with close to 100% viability by propidium iodide (data not shown). We conducted ATRA treatment experiments for 1–4 days and the earliest observed effect occurred after 1 day, and that cell death became prominent after 2 days. To activate TLR2/1, a synthetic 19-kDa lipopeptide derived from Mycobacterium tuberculosis (EMC Microcollections) was used, and to activate TLR4, purified LPS (Sigma-Aldrich) was used and prepared as previously described (13). P. acnes sonicate was prepared as previously described (2).

Isolation of monocytes
PBMC were isolated from human whole blood as previously described (13). Monocytes were enriched using Percoll (Amersham Biosciences) and adhered onto culture dishes for 2 h in RPMI 1640 containing 5% FBS (HyClone). The cells were washed with 1X PBS (Invitrogen Life Technologies) and the adherent cells were cultured as previously described (13).

Quantitative PCR (qPCR)
RNA was isolated from monocytes using TRizol Reagent (Invitrogen Life Technologies), and cDNA was synthesized using the iScript cDNA Synthesis Kit (Bio-Rad). The following primers were designed using Primer Express (Applied Biosystems): 36B4, 5'-CCA CGC TGC TGA ACA TGC T-3', 5'-CCA CGC TGC TGA ACA TGC T-3'.

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3 Abbreviations used in this paper: RAR, retinoic acid receptor; ATRA, all-trans retinoic acid; ΔMFI, mean fluorescence value; qPCR, quantitative PCR; TLR2/1L, TLR2/1 ligand; TLR4L, TLR4 ligand.

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TCG AAC ACC TGC TGG AC-3'; CD14, 5'-GCC TCC GATG CAT GTG-3'; 5'-AGC CCA GCG AAC GAC AGA-3'; and ABCA1, 5'-TGT CCA GTC CAG TAA TGG TGT TG-3', 5'-AAG CGA GATG GTC CGG ATT-3'. TLR primer sequences were previously published (14). SYBR Green reactions were conducted with the IQ SYBR Green mix (Bio-Rad). Reactions were run on the MJR Opticon Continuous Fluorescence detector (Bio-Rad) and analyzed with Opticon Monitor Software 1.08 (Bio-Rad). The relative quantities of the gene tested per sample were calculated against 36B4 using the ΔΔC(T) formula as previously described (15).

Flow cytometry

Cells were labeled for surface proteins using mAbs and analyzed by flow cytometry as previously described (13). We used Abs against TLR2 (2392; Genentech), TLR1 (eBioscience), TLR4 (eBioscience), CD14 (CalTag Laboratories), and CD40 (BD Pharmingen). We determined cell viability by propidium iodide labeling (BD Pharmingen).

Cytokine ELISAs

For the pretreatment experiments, monocytes were adhered to 24-well plates and treated with DMSO or ATRA for 24 h, before addition of the TLR2/1 ligand (TLR2/1L), TLR4 ligand (TLR4L), or P. acnes sonicate for 16 h, after which the supernatants were harvested. For the cotreatment experiments the incubations were done simultaneously. The supernatants were assayed for cytokines using the sandwich ELISA method with Ab sets for IL-12p40, IL-6, TNF-α (Biosource), and IL-8 (BD Pharmingen).

Results and Discussion

Although TLR activation is essential for host defense against microbial pathogens, the resulting inflammation can contribute to tissue injury. Given the role of TLR2 in mediating inflammation in acne, we hypothesized that one mechanism for the transcriptional regulation of TLR2 by ATRA is through an indirect method.

We also sought to determine whether ATRA had the ability to directly suppress TLR-mediated inflammatory responses.
Therefore, we simultaneously treated monocytes with TLR2/1L or TLR4L and either ATRA or carrier control, then assayed the cell supernatants for cytokine levels. Cotreatment with ATRA at the highest concentration of the ligands reduced both TLR2/1L- and TLR4L-induced IL-12p40 release by 42% and 44%, TNF-α by 56% and 49%, and IL-6 by 48% and 37%, respectively (Fig. 3a and data not shown). In all cases, ATRA treatment had no significant effect on the release of IL-8 or the up-regulation of CD40 (Fig. 3b and data not shown). These findings were in contrast to the pretreatment of monocytes with ATRA, which inhibited TLR2/1 but not TLR4L-induced cytokine responses. We investigated cell death during co-treatment of the TLR ligands and ATRA. Using propidium iodide, we labeled for dead cells and found no significant difference, with viability over 95% (Fig. 3c). These data suggest that cytotoxicity is not the primary mechanism responsible for the lower cytokine levels. The most likely explanation for the ability of ATRA to directly suppress TLR-mediated inflammatory responses is by affecting TLR signaling or mRNA stability. There is already evidence in the literature that retinoids inhibit TLR4 signaling by physical association of retinoid X receptor with NF-κB, thereby inhibiting IL-12p40 transcription (18), and that activation of NF-κB is a shared signaling pathway by all TLRs (1). Alternatively, retinoids have been reported to decrease mRNA stability (19).

Because P. acnes has been shown to induce inflammatory cytokines through activation of TLR2 but not TLR1 (2), we tested whether ATRA could reduce P. acnes-induced monocyte cytokine release. Monocytes were pretreated with ATRA, then activated with P. acnes sonicate or cotreated with ATRA and P. acnes simultaneously. ATRA pretreatment reduced P. acnes-induced release of both IL-12p40 and TNF-α by 53% and 67%, respectively at the highest P. acnes concentration tested (Fig. 4a). ATRA cotreatment resulted in a reduction of IL-12p40 and TNF-α levels by 37% and 31%, respectively (Fig. 4b). The levels of IL-6, IL-8, and CD40 were not affected with either pre- or cotreatment with ATRA (Fig. 4 and data not shown). These data demonstrate that ATRA is able to reduce P. acnes-induced inflammatory cytokine release, and therefore it is tempting to speculate that the ability of ATRA to modulate TLR2 expression and function accounts for the anti-inflammatory activity of retinoids during acne therapy. Commonly prescribed products containing ATRA as the active ingredient contain an equivalent concentration ranging from 6.67 × 10⁻³ M to 1.33 × 10⁻³ M, yet the concentrations of the retinoid that penetrate the epidermis are not known. The elucidation of the P. acnes ligand that activates monocytes via TLR2 should provide insight into the mechanism by which retinoids exert an anti-inflammatory effect in acne.
Our data provide evidence that one possible mechanism for the anti-inflammatory effect of ATRA is its ability to regulate TLR2 expression and activation. Other mechanisms may contribute to the anti-inflammatory effect of ATRA, yet in the absence of an animal model, it is difficult to determine which pathway is dominant. Nevertheless, the finding that a pharmacologic agent can inhibit TLR expression and function is novel and provides a rationale for developing other TLR antagonists as therapeutic agents. Clearly, it will be of interest to determine whether other anti-inflammatory agents exert their effect on TLR activation. Already, certain lipid A analogues have been reported to antagonize TLR2 and TLR4 activation through interaction with shared receptor components, resulting in loss of TNF-α secretion but not NO production in both transfected cell lines as well as primary human alveolar macrophages (20, 21). The data suggested that the most likely mechanism for the inhibition was through blocking the physical interactions of the ligand with the receptor components, distinct from the mechanism(s) by which ATRA inhibits TLR2-mediated responses.

Therefore, the ability of ATRA to inhibit TLR expression and activation provides a novel therapeutic approach to managing diseases in which TLR-induced inflammation contributes to tissue injury.

Disclosures

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References