All-*Trans* Retinoic Acid Shifts *Propionibacterium acnes*-Induced Matrix Degradation Expression Profile toward Matrix Preservation in Human Monocytes

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Propionibacterium acnes is a critical component in the pathogenesis of acne vulgaris, stimulating the production of various inflammatory mediators, such as cytokines and chemokines, important in the local inflammatory response found in acne. This study explored the role of *P. acnes* and its ability to induce matrix metalloproteinases (MMPs) in primary human monocytes and how this induction is regulated by retinoids. MMP-1- and MMP-9-expressing cells were present in perifollicular and dermal inflammatory infiltrates within acne lesions, suggesting their role in acne pathogenesis. *In vitro*, we found that *P. acnes* induced MMP-9 and MMP-1 mRNA, and the expression of MMP-9, but not of MMP-1, was found to be Toll-like receptor 2-dependent. *P. acnes* induced the mRNA expression of tissue inhibitors of metalloproteinase (TIMP)-1, the main regulator of MMP-9 and MMP-1. Treatment of monocytes with all-*trans* retinoic acid (ATRA) significantly decreased baseline MMP-9 expression. Furthermore, co-treatment of monocytes with ATRA and *P. acnes* inhibited MMP-9 and MMP-1 induction, while augmenting TIMP-1 expression. These data indicate that *P. acnes*-induced MMPs and TIMPs may be involved in acne pathogenesis and that retinoic acid modulates MMP and TIMP expression, shifting from a matrix-degradative phenotype to a matrix-preserving phenotype.

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INTRODUCTION

Activation of the innate immune system by cutaneous pathogens leads to a direct host defense response, yet the same mechanism is used by the pathogen to induce inflammation and tissue injury that leads to disease. In acne vulgaris, *Propionibacterium acnes* activates innate cells to induce a protective antimicrobial response (Vowels *et al.*, 1995) but also contributes to disease by releasing various inflammatory mediators including cytokines and chemokines that lead to tissue injury. The release of these pro-inflammatory mediators has been shown to be via a Toll-like receptor (TLR)2-dependent mechanism (Kim *et al.*, 2002).

Recently, TLRs have been implicated in mediating the expressions of various matrix metalloproteinases (MMPs) (Kyburz *et al.*, 2003; Gebbia *et al.*, 2004; Elass *et al.*, 2005). MMPs play a role in numerous inflammatory conditions, including rheumatoid arthritis (Burrage *et al.*, 2006) and atherosclerosis (Tayebjee *et al.*, 2005). In addition, there is evidence to support a role for MMPs in cutaneous microbial-based diseases, such as Lyme disease (Hu *et al.*, 2001) and tuberculosis (Elkington *et al.*, 2005). Increased levels of MMP-1, MMP-3, and MMP-9 have been shown to be present in acne lesions (Kang *et al.*, 2005; Papakonstantinou *et al.*, 2005; Trivedi *et al.*, 2006), but the role of MMPs in acne pathogenesis is not clear.

Given the potential role for MMPs in inflammation, tissue destruction, and scar formation (Kang *et al.*, 2005), we hypothesized that *P. acnes* may be an important stimulus for MMPs in acne. We sought to explore the mechanism of MMPs production, including the potential involvement of TLRs and how retinoid therapy modulates MMPs in acne.

RESULTS

MMP expression in acne lesions

To determine whether MMPs play a role in acne pathogenesis, we sought to localize the expression of MMP-1 and MMP-9 *in vivo*. Using immunohistochemistry, acne lesions were obtained from patients and stained for MMPs. In lesional skin, we found an abundance of MMP-1-expressing cells predominantly in the inflammatory infiltrate at the base of the pilosebaceous follicles (Figure 1). MMP-1 expression was also detected in the inflammatory cells within the

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Abbreviations: MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; TLR, Toll-like receptor

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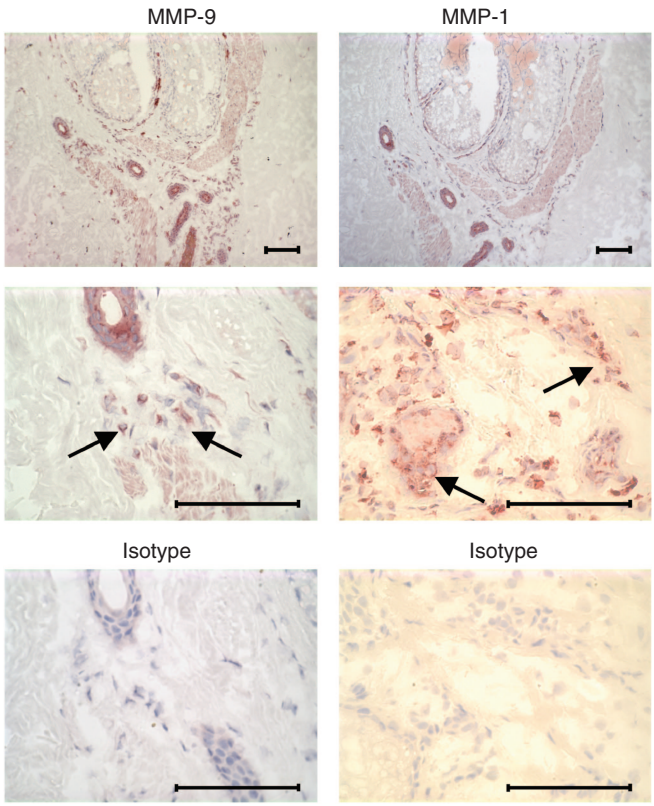

Figure 1. MMP-9 and MMP-1 expression in acne lesions. Representative sections from skin biopsy specimens from three acne patients with inflammatory lesions were stained by the immunoperoxidase method with mAbs specific for MMP-9 and MMP-1, or control antibodies, monoclonal IgG1 and IgG2a, respectively. Two patient samples are shown with MMP-expressing cells indicated by arrowheads. Samples from all three patients showed similar staining patterns for MMP-9 and MMP-1. Bars = 100 μm.

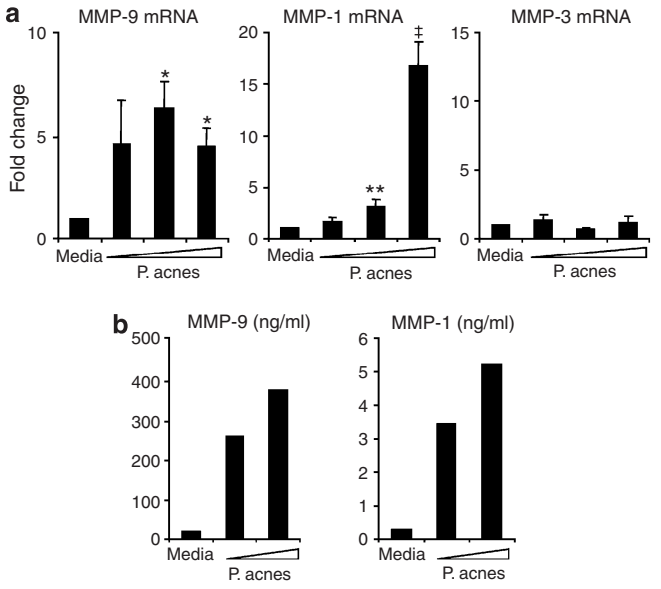

Figure 2. *P. acnes* induces MMP-9 and MMP-1 expression in monocytes, but not that of MMP-3. (**a**) Primary human monocytes were stimulated with various concentrations of *P. acnes* sonicate (0.01–1 μg ml^{-1}) and mRNA was harvested after 16 hours. mRNA levels of MMP-9, MMP-1, and MMP-3 were determined using quantitative RT-PCR and normalized to the housekeeping gene *36B4*. Data is expressed as fold change over media and is a composite of at least three independent experiments ± SEM. (**b**) Primary human monocytes were stimulated with various concentrations of *P. acnes* sonicate (0.01–1 μg ml^{-1}) and culture supernatants were harvested at 48 hours. Samples were concentrated 10-fold and MMP-9 and MMP-1 levels were determined by cytokine array (Pierce SearchLight Multiplex). Representative experiment, 1 of 3, is shown. (*P*-values: *$\leqslant$0.05, **$\leqslant$0.01, ‡$\leqslant$0.001).

papillary dermis and the dermal–epidermal junction. The expression of MMP-9 was noted to be similar, with the perifollicular inflammatory infiltrate staining positive for MMP-9 (Figure 1), although fewer in number than for MMP-1. All three patient samples showed similar staining patterns for both MMP-1 and MMP-9. In addition, the eccrine glands stained positive for MMP-1 and MMP-9. Interestingly, there was significant expression of MMP-9 in the upper layers of the epidermis, when compared to isotype-matched control. Sparse MMP-9 expression in single basal keratinocytes has been previously reported in normal skin samples (Narbutt *et al.*, 2006). This suggests that, in addition to the inflammatory infiltrate, keratinocytes may also be a source of MMP-9 *in vivo* in acne lesions.

P. acnes upregulates MMP-9 and MMP-1 gene expressions, but not that of MMP-3

Given that MMP-expressing cells were mainly inflammatory cells and various pathogens have shown to stimulate MMP production in monocytes, we hypothesized that MMPs may also be one of the mediators involved in *P. acnes*-induced inflammatory response in acne. To test this, we isolated monocytes from normal donors and determined relative mRNA levels of various MMPs, including MMP-1, MMP-3, and MMP-9, before and after stimulation with *P. acnes* sonicate. Primary human monocytes expressed low levels of MMP-1, MMP-3, and MMP-9 mRNA at baseline. Stimulation of monocytes with *P. acnes* resulted in marked induction of MMP-9 mRNA as measured by quantitative reverse transcriptase PCR (RT-PCR) at all concentrations tested (Figure 2a). Stimulation of monocytes by *P. acnes* also induced MMP-1 mRNA in a dose-dependent manner with a 16-fold change at the highest concentration tested (Figure 2a). Although MMP-9 and MMP-1 expression was upregulated, no significant increase in MMP-3 mRNA was induced by *P. acnes* (Figure 2a). Maximal upregulation of MMP-9 and MMP-1 transcripts was seen between 12 and 16 hours (data not shown).

Additionally, culture supernatants were analyzed at 48 hours after stimulation with *P. acnes* for MMP-9 and MMP-1 protein. MMP-9 and MMP-1 were found to be present in culture supernatants in a dose-dependant manner, in agreement with the mRNA data (Figure 2b). The presence of MMP-9 was approximately 100-fold greater in comparison with MMP-1. Therefore, our data suggest that stimulation of monocytes with *P. acnes* results in increased MMP-9 and MMP-1 mRNA and protein expression.