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Correlation of increased *PARP14* and *CCL26* expression in biopsies from children with eosinophilic esophagitis

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To the Editor

PARP14 is one of 18 poly-ADP ribosyl polymerase (PARP) family members that contain a catalytic domain conferring ADP-ribosyltransferase activity, and was initially identified as a transcriptional cofactor for signal transducer and activator of transcription (STAT)6 activity.[1] Cytokine-stimulated STAT6 DNA binding activates PARP14 catalytic activity, resulting in changes in target gene chromatin.[2] Because STAT6 plays an obligate role in the development of allergic inflammation,[1] and other PARPs including PARP1 may contribute to allergic inflammation,[3] we previously assessed the requirement for PARP14 in the development of allergic inflammation. In a model of allergic airway inflammation, mice deficient in PARP14 had diminished cellular infiltrates, increased lung function, and decreased T_H2 cytokine production compared with control mice.[4] Similarly, treatment of wild-type mice with PARP inhibitors during or after the development of disease resulted in decreased airway inflammation, T_H2 cell development, and increased lung function compared with control mice.[4] At least part of the mechanism of PARP14 function was through direct effects of PARP14 on T_H2 cytokine genes, and the T_H2 transcription factor *Gata3*.[4] However, the requirement for PARP14 in STAT6-dependent gene expression in nonlymphoid cells, and whether PARP14 expression is altered in human disease, has not been assessed.

We initially tested whether there are changes in the expression of members of the macro-PARP subfamily of *PARP* genes in children with eosinophilic esophagitis (EoE) compared with control samples. We obtained esophageal biopsies from children with EoE (Indiana University [IU] population; see at www.jacionline.org) and control samples from children who had esophageal biopsies for diagnostic purposes but did not have eosinophilic esophagitis. RNA was isolated from biopsies, and cDNA was assessed for gene expression by using quantitative PCR. We observed a 5.95-fold average increase in *PARP14*

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expression, a 3.1-fold average increase in *PARP1* expression, and a decrease in *PARP15* expression in EoE biopsies compared with controls (Fig 1, *A*). In contrast, there was no significant difference in the expression of *PARP9* (Fig 1, *A*).

To confirm this finding, we examined *PARP14* expression in a population from Cincinnati Children's Hospital Medical Center through the use of high-throughput RNA sequencing.[5] Compared with the IU population, this population had more severe inflammation[5]. Following analysis of the RNA-sequencing data, we observed similar (4.5-fold) increases in *PARP14* expression as seen in the IU population (Fig 1, *B*).

The chemokine CCL26 (Eotaxin-3) is a critical component of the pathology of EoE. *CCL26* expression is dramatically increased in biopsies from patients with EoE, and single nucleotide polymorphisms in the *CCL26* gene are associated with increased disease incidence.[6,7] Moreover, STAT6 regulates CCL26 in esophageal cells.[8] To determine whether *PARP14* expression correlated with *CCL26* expression, we tested the association of expression of these 2 genes in esophageal biopsies from patients with EoE and observed a strong correlation coefficient (IU population: $R = 0.81$; $P = .0002$, Cincinnati Children's Hospital Medical Center population: $R = 0.61$, $P = .03$) (Fig 1, *C*). In contrast, there was no significant correlation between *PARP1* and *CCL26* expression ($R = 0.30$, $P = .27$). There is significant heterogeneity in the expression of PARP14 in the biopsy samples, with some overlap in the control biopsy samples (Fig 1, *A*). This heterogeneity was lessened in samples from patients with more severe inflammation (Fig 1, *B*). Thus, PARP14 expression is increased and CCL26 and PARP14 gene expression is correlated in 2 populations interrogated by 2 distinct methods.

Because our results suggested a relationship between *PARP14* and *CCL26*, we tested the ability of PARP14 to regulate *CCL26* directly. The esophageal cell line TE-7 was transfected with a *CCL26* luciferase reporter vector and plasmids encoding STAT6 and/or PARP14 before incubation for 24 hours in the presence or absence of the STAT6-activating cytokines IL-4 and IL-13. Consistent with previous results, transfection of STAT6-expressing plasmids increased *CCL26* reporter activity (Fig 2, *A*). Moreover, transfection of PARP14 alone increased basal and cytokine-induced reporter activity (Fig 2, *A*). Importantly, cotransfection of STAT6 and PARP14 significantly increased *CCL26* reporter activity over cells transfected with STAT6 alone (Fig 2, *A*). The effects of PARP14 expression were entirely dependent on STAT6 binding because they were not observed when the plasmid was cotransfected with a *CCL26* reporter that had a mutation in the STAT6 binding site (Fig 2, *A*). These results suggested that PARP14 might be a viable target for modulating the expression of *CCL26*. To test this directly, TE-7 cells were incubated with IL-4 or IL-13 in the presence or absence of the PARP activity inhibitor PJ34 before expression of the endogenous *CCL26* gene was assessed. We observed that IL-4 and IL-13 increased *CCL26* mRNA and that incubation with the PARP inhibitor attenuated the induction in response to either cytokine (Fig 2, *B*). Similar results were observed in the TE-1 esophageal cell line. Thus, PARP14, and PARP activity, contribute to the regulation of *CCL26* in esophageal cells. These results do not exclude the possibility that PARP14 is expressed by, and functions in, additional cell types that contribute to EoE.

Although we are only beginning to understand the *in vivo* functions of PARP14, this report, coupled with our previous work,[4] suggests that PARP14 has a significant role in the development of allergic inflammation. It likely works in multiple cell types, including in T cells, where it results in increased T_H2 and T_H9 development,[4,9] and in target organ epithelial cells, enhancing the production of proallergic chemokines. Our results raise the possibility that targeting PARP14, or even PARP activity in general, might be an effective therapy for allergic diseases including EoE.

METHODS

Gene expression

RNA was isolated from the esophageal biopsies (IU population), and gene expression was assessed for the indicated genes by using quantitative PCR. The mRNA expression of *CCL26* was determined by using the ΔCt method. RNA isolated from the Cincinnati Children's Hospital Medical Center population was sequenced at the Cincinnati Children's Hospital Medical Center Gene Discovery and Genetic Variation Core as previously described.[5]

TE-7 esophageal epithelial cells were incubated in the presence or absence of IL-4 (2.5 ng/mL) or IL-13 (20 ng/mL), with or without the PARP inhibitor PJ34 (25 μM). CCL26 mRNA expression was assessed by using quantitative PCR.

Luciferase assay

TE-7 esophageal epithelial cells were transfected with a CCL26 luciferase reporter or a reporter carrying a mutation in the STAT6 binding site, and control, STAT6-, or PARP14-expressing plasmids. Cells were incubated in the presence or absence of IL-4 (25 ng/mL) or IL-13 (25 ng/mL) for 24 hours before luciferase activity was assessed.

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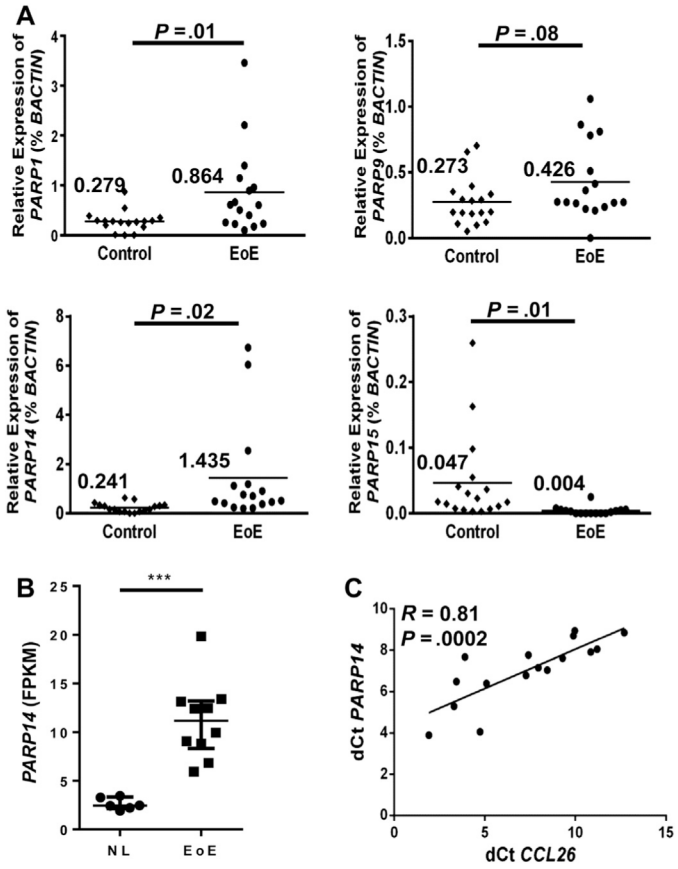

FIG 1.

Correlation of *PARP14* and *CCL26* expression. **A,** Gene expression was assessed for the indicated genes from IU population biopsies. Results are presented as percent of *BACTIN* control. **B,** *PARP14* expression in CCHMC population biopsies was determined by using RNA sequencing. **C,** The mRNA expression of *CCL26* was graphed against *PAPR14* expression (IU population). Significance was determined by using ANOVA. *CCHMC*, Cincinnati Children's Hospital Medical Center; *NL*, normal. ***$P < 10^{-3}$. *FPKM*, fragments per kilobase of transcript per million mapped reads.

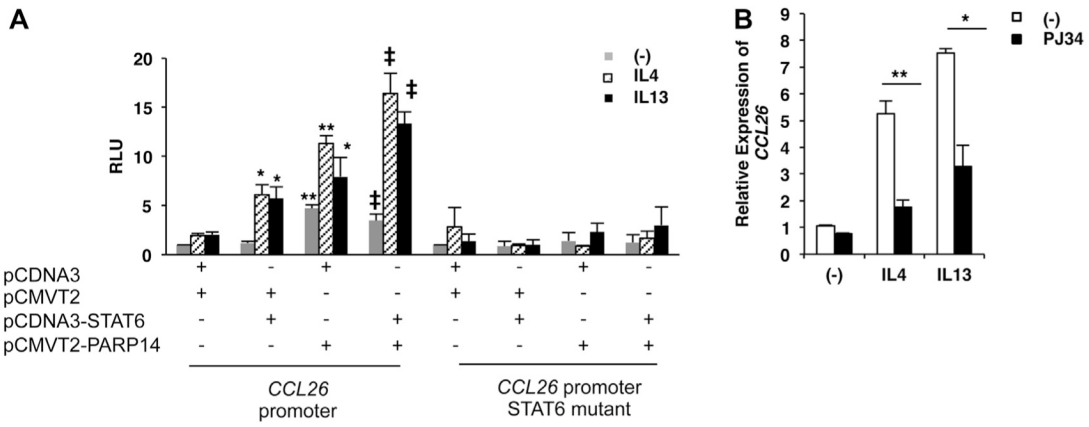

FIG 2.

PARP14 activates the CCL26 gene. **A,** *CCL26* promoter reporter activity with cotransfection of STAT6-and/or PARP14-expressing plasmids into TE-7 esophageal cells. **P* < .05; ***P* < .001, compared with control plasmid transfection; ‡*P* < .05 compared with STAT6 transfection. **B,** CCL26 expression in cytokine-stimulated TE-7 esophageal cells incubated with the PARP inhibitor PJ34. Results are the average of at least 3 experiments. **P* < .05, ***P* < .001, and ****P* < .005. *P* values determined by using the Student *t* test.