

Letter to the Editor

Omeprazole inhibits IL-4 and IL-13 signaling signal transducer and activator of transcription 6 activation and reduces lung inflammation in murine asthma

To the Editor:

Proton pump inhibitors (PPIs), which are the cornerstone of gastroesophageal reflux disease therapy, have been reported to

improve asthma and eosinophilic esophagitis (EE) in patients with associated gastroesophageal reflux disease symptoms.^{1,2} The most accepted explanation for these findings is based on the idea that pathologic acidic reflux can act as a triggering factor for these diseases through proximal extent and laryngopharyngeal reflux in asthma and impairment of the epithelial barrier in EE. Thus, it is believed that acid suppression can prevent these pathogenic mechanisms. Nonetheless, a number of findings suggested the possibility that omeprazole could have a direct effect in

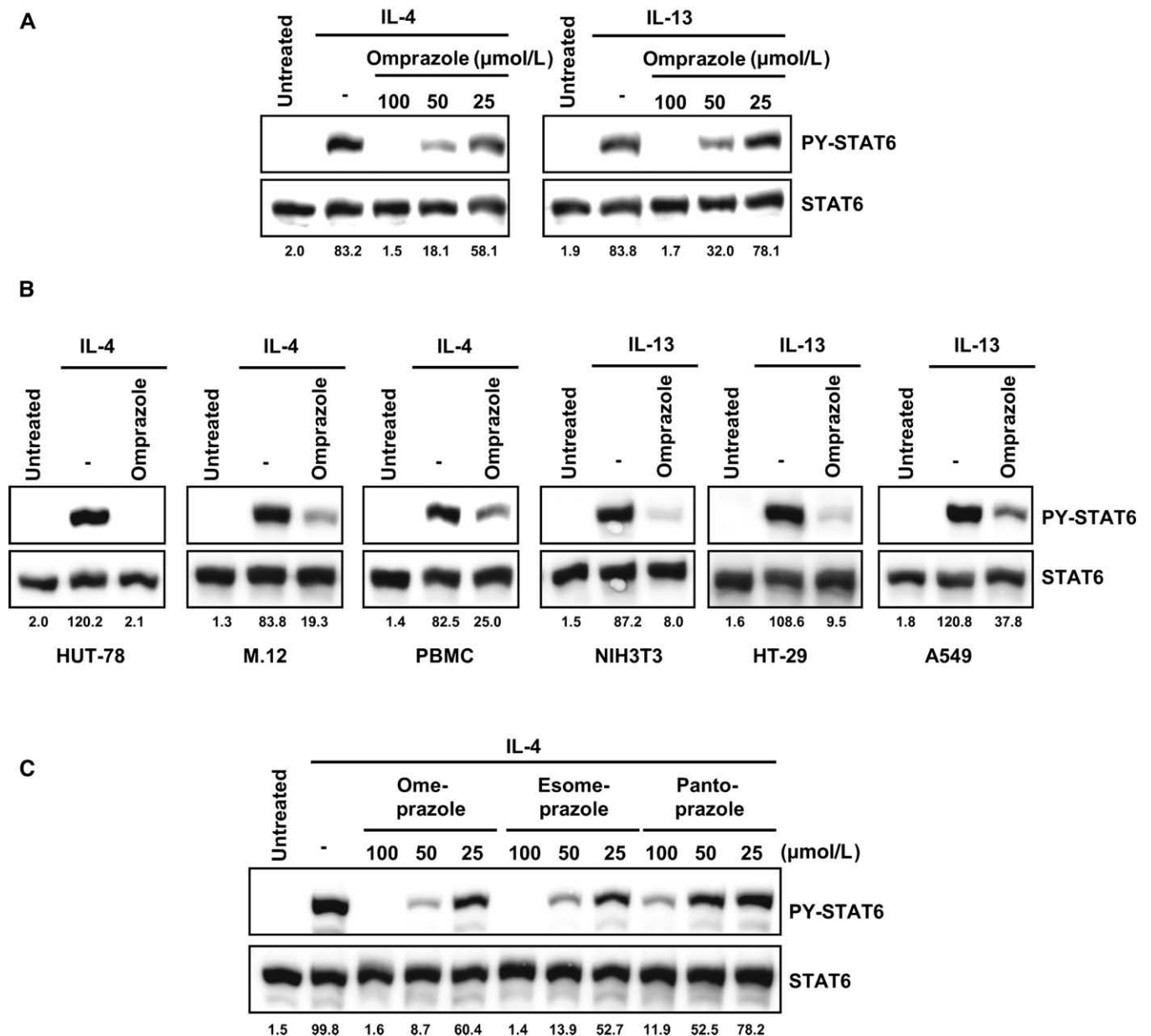


FIG 1. Inhibition of STAT6 activation by PPIs. HL-60 (**A** and **C**) and other cells (**B**) were preincubated with PPIs before cytokine stimulation (10 ng/mL). STAT6 phosphorylation (PY-STAT6) and total protein (STAT6) were then determined. **B**, Omeprazole 50 μmol/L was used. Numbers under blots indicate relative phosphorylation ([density phosphorylated bands/density total protein bands] × 100). Data are representative of 3 independent experiments.

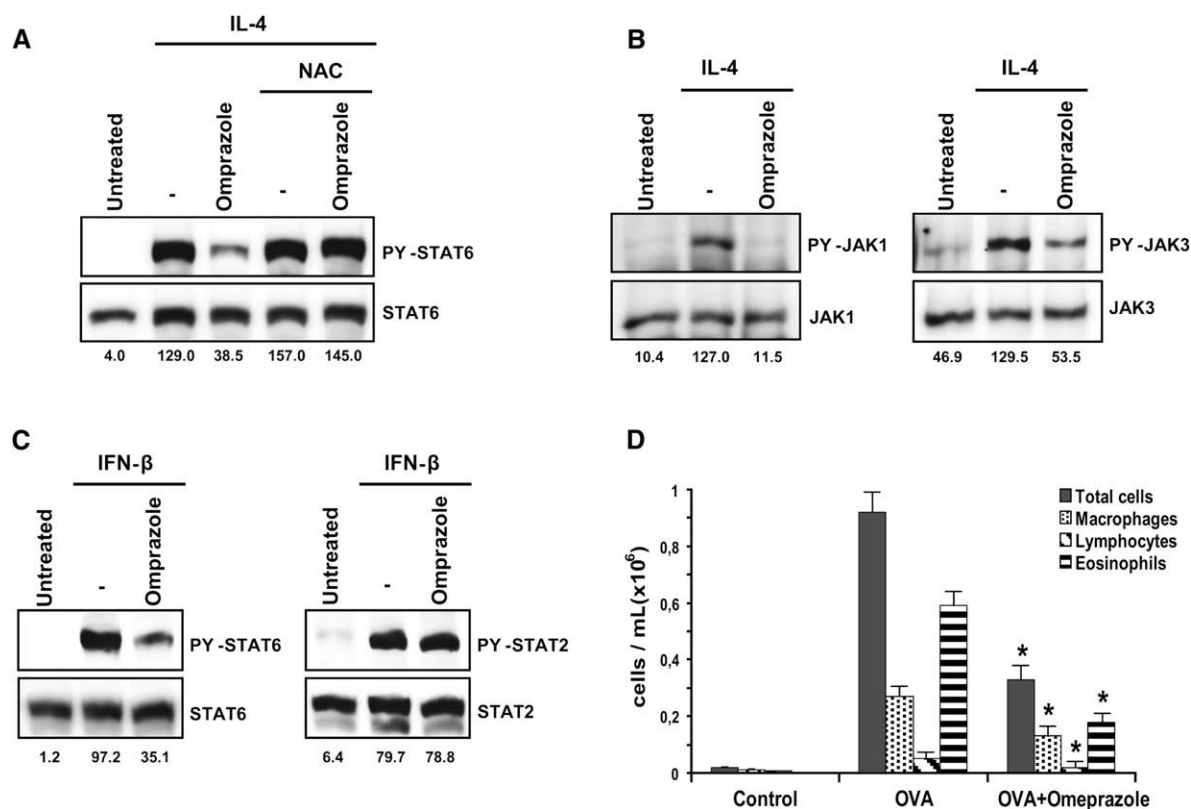


FIG 2. Mechanism and biological effects of STAT6 inhibition. Phosphorylation of STAT6 (**A** and **C**), JAKs (**B**), and STAT2 (**C**) in the absence or presence of omeprazole (50 μ M/L). **A**, N-acetyl-cysteine (NAC) was used at 5 mmol/L. The relative phosphorylation was calculated as in Fig 1. **D**, Inflammatory cells in bronchoalveolar lavage fluid (BALF) from control nonasthmatic mice ($n=6$), mice with asthma induced by ovalbumin without treatment (OVA, $n=6$), or treated with omeprazole (OVA+Omeprazole, $n=6$). * P value $<.05$ versus asthmatic mice without omeprazole treatment. Data are representative of 3 independent experiments.

molecular pathways involved in asthma and EE: (1) the inhibitory mechanism of omeprazole in gastroesophageal reflux disease implies alkylation of cysteine residues in gastric H⁺/K⁺-adenosine triphosphatase,^{3,4} (2) asthma and EE are prototypic T_H2 diseases in which the cytokines IL-4 and IL-13 play a principal role through the activation of the transcription factor signal transducer and activator of transcription 6 (STAT6),^{5,6} and (3) we have recently demonstrated that some chloromethyl ketones can downregulate STAT6 by mechanisms involving cysteine alkylation.⁷

On the theoretical basis that cysteine reactivity of omeprazole may affect the regulation of STAT6, we analyzed its effect in the activation of STAT6 by IL-4 and IL-13. To this end, omeprazole (Sigma-Aldrich, St Louis, Mo) was first activated for 30 minutes in culture media at pH 5.5 to convert to the active sulfenamide form.^{3,4} Activated omeprazole was then mixed with an equal volume of media containing cells at neutral pH and incubated for 1 hour before stimulation with cytokines for 20 minutes. The amount of omeprazole employed was that reported to show *in vitro* activity.³ Thus, treatment of cells with omeprazole inhibited the phosphorylation of STAT6 induced by IL-4 and IL-13 in a dose-dependent manner (Fig 1, A). The inhibitory effect of omeprazole seemed to be universal because it was observed in primary PBMCs and multiple cell lines of myeloid (HL-60), lymphoid (HUT-78, M.12), fibroblast (NIH3T3), lung (A549), and colon (HT-29) origins (Fig 1, B). Cell viability was not affected under the experimental conditions described, even for longer cultures (data not shown). Analysis of other PPIs indicated that

esomeprazole (AstraZeneca, London, UK) had a similar effect, whereas pantoprazole (Nycomed, Zurich, Switzerland) showed a lower inhibitory activity (Fig 1, C). These differences may be explained by the fact that the rate of conversion to the active sulfenamide and the reactivity with cysteines are lower in pantoprazole.⁴

As expected from their mechanisms of action,⁴ the effect of PPIs in the activation of STAT6 was likely mediated by their alkylating activity because N-acetyl-cysteine blocked the inhibitory effect of omeprazole (Fig 2, A). However, in contrast with our previous findings showing proteolytic regulation of STAT6 by alkylating chloromethyl ketones,⁷ PPIs did not provoke STAT6 protein loss (Fig 1), indicating that their inhibitory activity should be mediated through alternative molecular mechanisms. This was supported by the observation that treatment of cells with omeprazole also inhibited upstream events like the activation of janus kinase 1 (JAK1) and JAK3 by IL-4 (Fig 2, B), suggesting that PPIs would target early proteins involved in IL-4/IL-13 signaling. In this regard, it is tempting to hypothesize that PPIs may inhibit IL-4/IL-13 signaling by reacting with cysteine residues in the common IL-4 receptor α -chain because their cationic active forms are relatively membrane-impermeable.⁴ However, we could not exclude a modification of STAT6 by PPIs. This was based on the fact that omeprazole inhibited the phosphorylation of STAT6 but not STAT2 induced by IFN- β (Fig 2, C), indicating a specificity for STAT6 that correlated with our previous observations showing downregulation of STAT6 but not STAT2 by

alkylating chloromethyl ketones.⁷ In this case, it may be possible that modification of proteins by PPIs would not be enough to alter multiprotein complexes containing STAT6 to allow the action of proteases as proposed for chloromethyl ketones,⁷ although these modifications may impede the interaction of STAT6 with IFN receptors, blocking its phosphorylation.

The inhibition of IL-4 and IL-13 signaling STAT6 activation has been proposed for the management of allergic diseases, as demonstrated by the efficiency of a soluble form of IL-4 receptor in the management of asthma.⁵ Accordingly, we found that omeprazole diminished the inflammatory response in a murine model of allergic asthma. To perform these experiments, animals were sensitized by intraperitoneal injection of 25 µg ovalbumin in PBS/alum (Sigma-Aldrich) and then challenged by ultrasonic nebulization with a solution of 1% ovalbumin in PBS on days 14 and 17. At day 21, animals were anesthetized and bronchoalveolar lavage fluids collected before fixing the lungs for histochemical analysis. Where indicated, omeprazole was orally administered on days 15 to 20 (40 mg/kg/d). In this case, omeprazole may convert to its active form in the acidic environment of asthmatic lung airways,⁸ facilitating its inhibitory activity. Thus, the presence of inflammatory cells, including eosinophils, was significantly reduced in bronchoalveolar lavage fluid (Fig 2, D) and lung sections (see this article's Fig E1 in the Online Repository at www.jacionline.org) from omeprazole-treated animals. These findings supported the proposal that the beneficial effects of PPIs in asthma may be related to their ability to inhibit IL-4/IL-13 signaling STAT6 activation. It has recently been reported that esomeprazole did not improve asthma in patients with asymptomatic gastroesophageal reflux and poorly controlled asthma.⁹ However, this study was focused in a subset of patients with asthma who were also refractory to other treatments. Thus, it may be possible that another group of patients could benefit from PPI therapy, as shown in other studies¹ and this murine model. It would be interesting to investigate what subsets of patients with asthma might benefit from PPI therapy.

In this report, we show a novel effect of omeprazole and other PPIs in inhibiting IL-4 and IL-13 signaling STAT6 that correlated with a reduction of inflammation in a murine model of asthma. Our findings raise the possibility that the beneficial effects of omeprazole in asthma and EE may be mediated, at least in part, through the inhibition of these pathways. The novel effects of

PPIs described in this study should merit further consideration in allergies but also in other diseases in which these cytokines play an active role.

Jose R. Cortes, BSc^a

Maria D. Rivas, PhD^a

Javier Molina-Infante, MD^b

Maria A. Gonzalez-Nuñez, MD^c

Moises Perez-G, BSc^a

Juan F. Masa, MD^d

Juan F. Sanchez, MD^e

Jose Zamorano, PhD^a

From ^aUnidad de Investigación, ^bServicio de Digestivo, ^cServicio de Anatomía Patológica, ^dServicio de Neumología-Centro de Investigación Biomedica en Red de Enfermedades Respiratorias (CiberRes), and ^eServicio de Medicina Interna, Hospital San Pedro de Alcantara, Cáceres, Spain. E-mail: jose.zamorano@ses.juntaextremadura.net.

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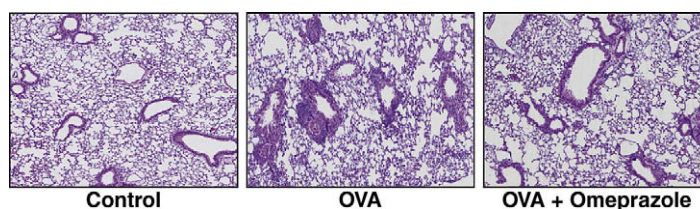


FIG E1. Omeprazole diminished asthma inflammation. Inflammatory infiltrating cells were detected by hematoxylin-eosin staining of lung sections from control nonasthmatic mice ($n = 6$), mice with asthma induced by ovalbumin without treatment (*OVA*, $n = 6$), or mice treated with omeprazole (*OVA + Omeprazole*, $n = 6$). Data are representative of 3 independent experiments.