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Remission of food allergy by the Janus kinase inhibitor ruxolitinib in mice☆,☆☆

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ABSTRACT

To clarify the role of Janus kinase (JAK) in and the efficacy of JAK inhibitors on food allergy, we investigated the effect of the clinically available JAK inhibitor ruxolitinib on mouse food allergy and the functions of cultured mast cells *in vitro*. Anaphylactic symptoms including diarrhea and decreases in body temperature pursuant to oral ovalbumin (OVA) challenges in food allergy mice were attenuated by the daily oral administration of ruxolitinib. This drug inhibited increases in mouse mast cell protease-1 concentrations in the serum and mast cell numbers in the intestines of these mice as well as degranulation, IL-13 production, and the spontaneous and IL-9-dependent survival of mouse bone marrow-derived mast cells in spite of the absence of an effect of ruxolitinib on passive systemic anaphylaxis. Anti-OVA IgG2a, IgE, and IgG1 serum levels and the release of IFN-γ, IL-4, IL-9, and IL-10 from the OVA-restimulated splenocytes of food allergy mice were also decreased by the treatment. Moreover, ruxolitinib administration to mice that had already exhibited anaphylactic responses to previous challenges reduced anaphylactic responses to further oral OVA challenges, which suggested that ruxolitinib has a therapeutic potential on food allergy. Our results showed that ruxolitinib remitted food allergy in mice mainly through immunosuppression and the prevention of mast cell hyperplasia, and partially through the inhibition of mast cell activation. We consider JAK inhibition to be a promising strategy for the prevention of food allergy, and ruxolitinib along with its derivatives inhibiting JAK as good candidates for therapeutic drugs to treat food allergy.

1. Introduction

Food allergy (FA) is a potentially life-threatening allergic disease triggered by the ingestion of a food allergen [1,2]. Many mild to severe symptoms including urticaria, vomiting, diarrhea, dyspnea, tachycardia, and anaphylactic shock are typically induced within minutes to hours after the intake of a food allergen. The prevalence of patients with FA has been increasing [3], and currently affects 8% of children [4] and 2% of adults [5]. FA causes 150 to 200 fatalities per year in the United States [6], even though established effective symptomatic treatments including an epinephrine injection are available [2]. A causal treatment to overcome this disease has not yet been established; therefore, the removal of allergens from the diet is the only reliable method to prevent FA.

Intestinal mast cell (MC) hyperplasia [7,8], Th2 sensitization against allergens [7,9], and IgE-dependent MC activation in acute allergic responses [7,10] were found to be important for the development of FA in both humans and a mouse model. Since MCs have been implicated in both allergic diathesis and the induction of acute allergic responses upon allergen exposure, compounds that modulate MC functions in and improve the pathological conditions of FA may represent potential candidates for therapeutic drugs used as alternatives to definitive treatments for FA.

Janus kinase (JAK) is a tyrosine kinase that participates in intracellular signal transduction pathways from the receptors of cytokines and growth factors to the transcriptional factor signal transducer and activator of transcription (STAT) to produce cellular responses [11]. Although many studies have demonstrated that JAK controls the cell viability, growth, and functions of MCs [11], the individual roles of each JAK in various conditions have not yet been determined. Two clinical JAK inhibitors, ruxolitinib (Jakafi, Incyte Co.) [12] and tofacitinib (Xeljanz, Pfizer Inc.) [13], were recently developed and have been used to treat myelofibrosis [14] and rheumatoid arthritis [15], respectively. Since the survival and activation of MCs are controlled by JAK, we investigated the effect of the JAK inhibitor ruxolitinib on a mouse FA model to examine the potential of the drug to treat FA and the importance of JAK in FA, with a focus on MC functions and immune responses. A passive systemic anaphylaxis (PSA) model and *in vitro* MC cultures were also used to clarify in detail the effect of ruxolitinib on MC functions.

Abbreviations: BMMCs, bone marrow-derived mast cells; FA, food allergy; JAK, Janus kinase; MCs, mast cells; MMCP, mouse mast cell protease; OVA, ovalbumin; PSA, passive systemic anaphylaxis; STAT, signal transducer and activator of transcription; Tr, regulatory T cell type.

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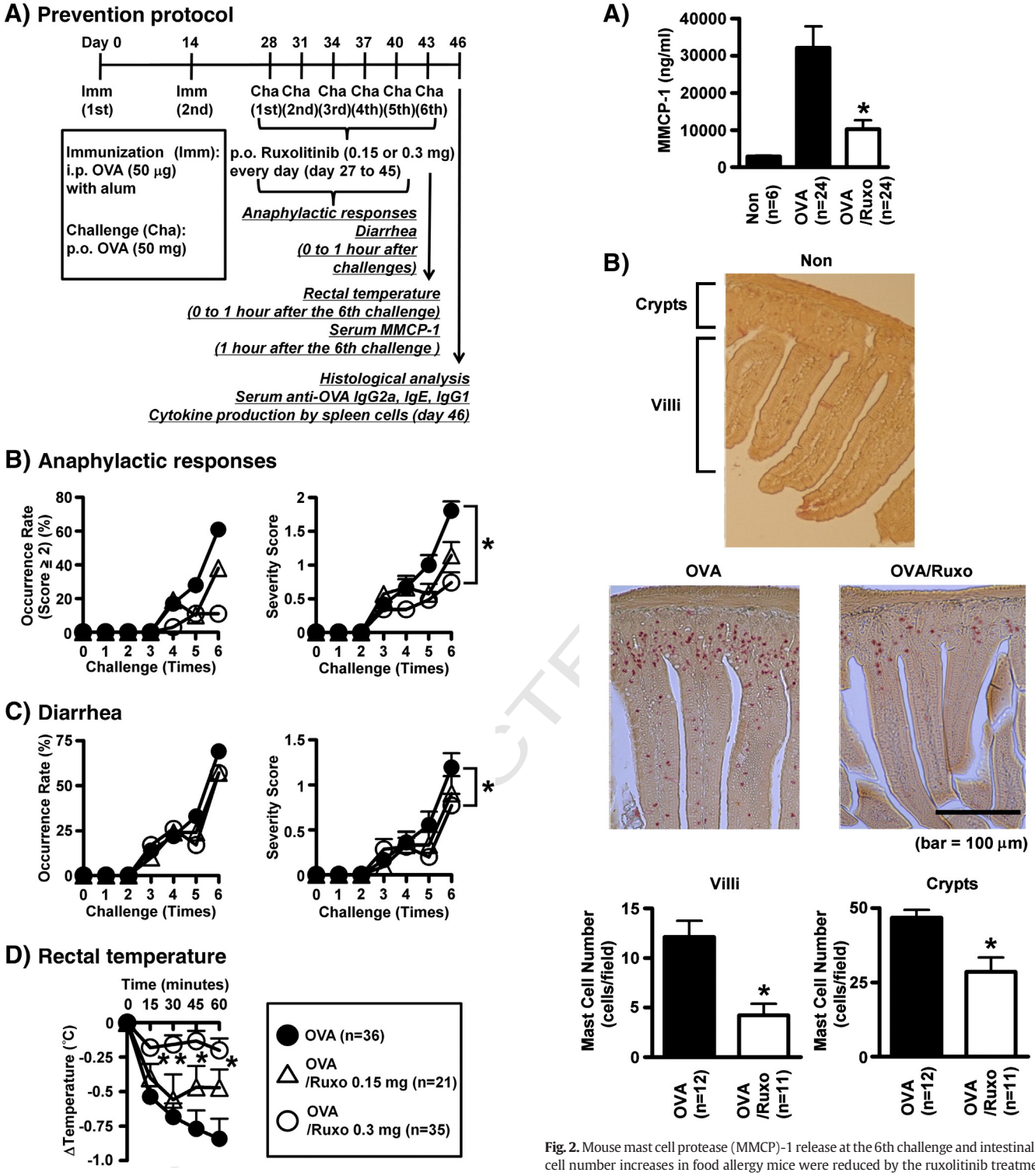

Fig. 1. Ruxolitinib in the prevention protocol reduced the induction of the symptoms of food allergy in mice due to ovalbumin (OVA) challenges. To examine the preventive effect of ruxolitinib (Ruxo), it was orally administered to immunized mice from the day before the 1st challenge to the day before sacrifice (A). The occurrence rates and severity scores of anaphylactic responses (B) and diarrhea (C) were calculated from monitoring within 1 h of the OVA challenges. Rectal temperatures were measured for 1 h after the 6th challenge (D). Symbols show values (occurrence rates in B and C) or means + SEM (severity scores in B and C, and D). Data were accumulated from three independent experiments. * $p < 0.05$, the OVA group ($n = 36$) *versus* the OVA/Ruxo 0.3 mg ($n = 35$) at the 6th challenge (Mann–Whitney U-test).

Fig. 2. Mouse mast cell protease (MMCP)-1 release at the 6th challenge and intestinal mast cell number increases in food allergy mice were reduced by the ruxolitinib treatment in the prevention protocol. (A) MMCP-1 serum concentrations in vehicle- or ruxolitinib (Ruxo, 0.3 mg)-treated food allergy mice were measured 1 h after the 6th challenge. Bars show means + SEM. Data were accumulated from two independent experiments. * $p < 0.05$, the ovalbumin (OVA) group ($n = 24$) *versus* the OVA/Ruxo ($n = 24$) (Tukey's post hoc test after one-way ANOVA among non ($n = 6$), OVA, and OVA/Ruxo groups). (B) Intestinal mast cells in the villi and crypts stained in red were counted to evaluate intestinal mast cell hyperplasia, an indicator of a predisposition for food allergy. Bars show means + SEM. Data were accumulated from two independent experiments. * $p < 0.05$, the OVA group ($n = 12$) *versus* the OVA/Ruxo ($n = 11$) (Mann–Whitney U-test).

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