Abundant Expression of c-Jun in Guinea Pig Sympathetic Ganglia Under Basal Conditions and Allergen Challenge

D. A. Groneberg,1,2 C. Peiser,1 Q. T. Dinh,2 J. Springer,3 and A. Fischer1

1Clinical Research Unit of Allergy, Department of Pediatric Pneumology and Immunology, Charité Medical School, Humboldt University of Berlin, Augustenburger Platz 1, 13353 Berlin, Germany
2Department of Medicine, Charité Medical School, Humboldt University of Berlin, Schumannstr. 20/21, 10117 Berlin, Germany
3Biomedical Research Center, Charité Medical School, Humboldt University of Berlin, Berlin, Germany

Abstract. Airway hyperresponsiveness, a keystone of allergic asthma, is mediated by the extrinsic airway innervation. As pathophysiological stimuli can induce the expression JUN proteins, which belong to the immediate early gene (IEG) family of transcription factors, the expression of c-Jun was examined under basal conditions and allergen challenge in guinea pig paravertebral and prevertebral sympathetic ganglia by quantitative double-labeling immunohistochemistry. C-Jun immunoreactivity was seen in 78.4 ± 3.5% under normal and 82.6 ± 4.6% under allergen-challenged conditions of protein-gene product (PGP) 9.5-positive sympathetic neurons of guinea pig superior cervical ganglia and 73.1 ± 2.8% (normal) and 76.1 ± 3.5% (allergen) ofstellate ganglion neurons. In the coeliac-superior mesenteric ganglion, 59.5 ± 5.0% (normal) and 57.5 ± 4.4% (allergen) of the PGP 9.5-positive sympathetic neurons were labeled for c-Jun. The high basal levels of c-Jun expression indicate that the presence of c-Jun is not exclusively related to noxious stimulation such as allergic airway inflammation in the guinea pig.

Key words: Allergic asthma — Guinea pig — c-Jun — Immunohistochemistry

Introduction

The chronic inflammatory airway disease asthma is characterized by bronchial hyperresponsiveness, infiltration of the airways submucosa with inflammatory
cells such as eosinophils and T cells, and mucus hypersecretion [1]. There are different states of asthma which are characterized by clinical and lung function parameters [2]. Different mechanisms have been demonstrated to play a major pathophysiologica role in the development and progression of the disease. Apart from underlying alterations in the immune system [3, 4], changes in airway innervation modulate the keystone of the disease bronchial hyperresponsiveness [5].

The immediate early gene (IEG) c-Jun or the related protein c-Fos, which together form the activator protein (AP-1) complex, were reported to be expressed in the nervous system after strong or noxious stimuli such as electrical stimulation, inflammation, axotomy, or pain [6]. In untreated animals, a basal expression of c-Jun in the central nervous system has been reported [7]. Most of the basal expression in the rat was seen in somatic and visceral motor areas. Apart from their expression in brain tissues [8–10], the expression of IEG transcription factors was also found in neurons of the enteric nervous system [11] and dorsal root ganglia [12].

In the present study, the expression of c-Jun in guinea pig sympathetic ganglia of normal and allergen-sensitized and challenged animals was addressed to elucidate changes in the expression pattern of immediate early genes in the neuronal component of allergic airway disease.

Materials and Methods

A total of 12 male Dunkin-Hartley guinea pigs were used. Animals were euthanized by inhalation of 100% CO₂. Six animals were ovalbumin-sensitized and challenged as described elsewhere [13] and 6 untreated animals were used as control group. In brief, the guinea pigs received intraperitoneal injections of 10 mg ovalbumin (Sigma, Munich, Germany), pertussis vaccine (250 ml, Berna, Berne, Switzerland), and aluminium hydroxide (50 mg, Sigma) at days 1, 14, and 28. At day 35, the animals were allergen-challenged by nebulization of 10 ml ovalbumin solution (0.1%) into a 4 L chamber over 60 min using a pressure nebulizer (pari-boy, Pari-Werk, Starnberg, Germany). Animals were sacrificed 24 h after allergen challenge, as this time point was previously demonstrated to be associated with an increase of tachykinins [13].

For Immunohistochemistry, the chest was opened, the right ventricle incised, and the animals were perfused through the left ventricle and ascending aorta with a rinsing solution containing 0.9% NaCl, 2.5% polyvinylpyrrolidion, 0.5% procaine hydrochloride, and 5000 U/L heparin to prevent blood clotting. This was followed by perfusion fixation with 4% buffered paraformaldehyde [14]. The different ganglia were explored and removed in toto, washed repeatedly, and stored overnight in 0.1 M phosphate buffer containing 18% sucrose for cryoprotection. Tissues were mounted on filter paper in optimum cutting temperature (OCT) compound and frozen in liquid nitrogen.

Immunohistochemistry was carried out as described elsewhere [15, 16]. In brief, 8 μm cryostat sections were washed several times in PBS and preincubated for 1 h at room temperature with 0.1 M phosphate buffer containing 1% bovine serum albumin and 10% normal swine serum. Sections were then rinsed in PBS and subjected to double-labeling immunohistochemistry by overnight incubation with rabbit polyclonal c-Jun antiserum and the mouse monoclonal neuronal marker Protein Gene Product 9.5 to identify the neurons within the ganglia (PGP 9.5, diluted 1:160) (Biotrend, Cologne, Germany). The c-Jun (dilution 1:1500) antiserum was characterized earlier and its specificity was demonstrated by in vivo preabsorption experiments [17, 18] and in vitro immunoprecipitation [18].

As secondary antibodies, biotinylated goat anti-rabbit IgG (Amersham, Braunschweig, Germany, 1:200) for the c-Jun and fluorescein isothiocyanate-labeled antimouse IgG (Amersham, 1:50) for the PGP 9.5 antibody were used for 1 h incubation at room temperature. The anti-rabbit antiserum was