Various TLR antagonists treatment effects on the development of allergen-specific responses

Glorian J. Crunt

Respiratory Diseases Research, Boehringer Ingelheim Pharma, Germany

*Corresponding Author : Glorian J. Crunt, Respiratory Diseases Research, Boehringer Ingelheim Pharma, Biberach an der Riss, Germany

Received : September 09, 2023
Accepted: September 10, 2023
Published : October 14, 2023

Abstract

Human trials are currently being conducted to determine whether certain Toll-like receptor (TLR) antagonists can improve the effectiveness of specific immunotherapy (SIT). According to recent clinical findings, this could be accomplished by boosting Th1 responses that are specific to allergens. Which TLR agonist is most appropriate for use in conjunction with SIT remains unclear, though. In a preclinical environment, we examined the capacity of five TLR antagonists—LTA, poly(I:C), LPS, R848, and CpG-ODN—activating TLR2, 3, 4, 7, and 9 to elicit allergen-specific Th1 and inhibit allergen-specific Th2 responses. Mice received two OVA aerosol challenges after receiving an intraperitoneal injection of ovalbumin (OVA)/Al(OH)3 along with agonists at several doses (0.0025, 0.025, 0.25, and 2.5 mg/kg). The outcomes of these studies demonstrated that the agonists and dose utilised were related to the activation of Th1 responses and the suppression of allergen-specific Th2 responses. With the exception of poly(I:C), all TLR agonists raised allergen-specific IgG2a and decreased allergen-specific IgE levels in the serum. In mice, the administration of LPS or CpG in conjunction with OVA/Alum also inhibited allergic cutaneous anaphylaxis. CpG and poly(I:C) elicited the strongest Th1 responses, as evidenced by the highest OVA-specific IgG2a levels in serum and the presence of IFN-γ in the BAL. According to this study, the greatest inhibitory effects on the development of allergen-specific Th2 responses in mice are found with TLR4 antagonist LPS and TLR9 agonist CpG-ODN.

Keywords : Asthma; TLR-Agonists; Innate-Inflammation; Inhibition

Introduction

Atopic asthma is brought on by the lung’s allergic immune system reacting to common environmental antigens. Long-acting β-agonists, oral or inhaled steroids, and leukotriene modifiers (Montelukast) are the most significant and commonly used treatments for asthma. Moreover, those with severe atopic asthma are treated with anti-IgE medication. In asthma or other atopic disorders, none of these medications have disease-modifying effects [1,2] and must be used consistently. As of right now, allergen-specific immunotherapy (SIT) is the only proven disease-modifying treatment for allergy sufferers [3]. Increasing doses of various allergens—typically standardised extracts of the allergen—are applied subcutaneously (SCIT) or sublingually (SLIT) over a maximum of three to five years. It’s unclear exactly how SIT mediates protective benefits through certain systems. According to recent research, it may be linked to elevated IgG4 levels specific to allergens, the development of immunological tolerance, or a little deviation in the Th2 responses specific to allergens directed towards Th1 [6, 7]. SIT has only modest benefits for treating patients with asthma, despite being beneficial in treating minor allergic reactions [8]. especially if the patient has allergies to a variety of substances. Allergen with adjuvants is the combination that is currently being worked on to increase the effectiveness of SIT and reduce treatment duration. Clinical tests using alum, MLP, virus-like particles, and CpG-ODN fused to allergen have all been successful.

Materials and Methods

Mice

We bought female Balb/c mice from Charles River in Sulzfeld, Germany. Animals were kept in an isolation facility under non-conventional settings. The mice were 8–12 weeks old when the tests started. The care and use of experimental animals was carried out in accordance with the regulations set forth by the
local and federal authorities in all experiments.

**Opponents of TLR**
The corresponding agonists, lipoteichonic acid from Staphylococcus aureus, LTA-SA, synthetic analogue of double stranded RNA, poly(I:C), lipopoly-saccharide from E. coli K12, LPS-EK, small synthetic antiviral imidazoquinoline compound, R848, and synthetic oligodeoxynucleotides containing unmethylated CpG dinucleotides, ODN1826, were used to activate the murine TLR2, TLR3, TLR4, TLR7, and TLR9. Every TLR agonist was acquired from InvivoGen, located in San Diego, USA.

**Protocols for Treatment**
On days 1, 14, and 21, 20 µg of OVA (Serva, Heidelberg, Germany) were intraperitoneally (i.p.) sensitised in 200 µl of 0.9% NaCl, which was then adsorbed to Al(OH)3 (Pierce, Rockford, USA). Only saline and Al(OH)3 were given to the negative controls. Mice were challenged with 1% OVA aerosol for 20 minutes on days 26 and 27. Intraperitoneally, TLR-agonist and OVA/Al(OH)3 were given on days 0, 14, and 21. After the previous challenge, 24 hours later, mice were slaughtered.

**Clearing the Bronchoalveoli**
On day 28, 24 hours following the final OVA challenge, the animals were slaughtered, their trachea were cannulated, and a bronchoalveolar lavage (BAL) was carried out according to the previously mentioned protocol.

**Cytokine Detection**
Following the manufacturer's instructions, mouse 22 plex cytokine/chemokine multiplex assays (LINCOplex, Millipore, St. Charles, or Multiplex, Meso Scale Discovery, Gaithersburg, USA) were used to determine the presence of cytokines and chemokines in the BAL fluid.

**Measurement of Allergen-Specific Immunoglobinulin**
After the previous challenge, blood samples were taken 23 hours later. The samples were centrifuged for 20 minutes at 14,000 rpm after being incubated for 30 minutes at room temperature. The obtained supernatant was frozen. IgE and IgG2a specific to OVA were determined using a conventional ELISA technique. To cover the plates, 100 ug/ml of OVA (Serva, Heidelberg, Germany) was utilised. The OVA-coated plates were then treated with serial dilutions of the various samples. Then, biotin rat anti-mouse IgE (BD Biosciences, Erembodegem, Belgium), IgE anti-tibody standard (Serotec Oxford, England), anti-OVA chicken (Dianova, Asker, Norway), and anti-mouse IgG2a biotin (BD Biosciences, Erembodegem, Belgium) were used to detect the antibodies binding to the OVA.

**Anaphylaxis of the Cutanes**
Mice that were standardised and challenged for active cutaneous anaphylaxis were given an intravenous (i.v.) injection of 200 µl of 1% Evans blue. Following isoflurane (3%–4% in pressurised air) anaesthesia, 5 µl of PBS containing 5 µg OVA was injected intradermally (i.d.) into the right ear of the mice. In the left ear, the negative controls were given 5 µl of PBS intravenously. Tissue samples were incubated with 300 µl formamide at 65˚C for 24 hours at 450 rpm on a shaker in order to extract colour. After dye extraction, a photometer was used to measure the concentration at 620 nm wavelength.

**The Historical Perspective**
The lungs were imbedded in paraffin wax after being preserved for 24 hours in 4% formalin. Slabs of the lungs, 2–3 µm thick, were stained according to normal histopathological procedures. Periodic acid-Schiff (PAS) reagents (Sigma-Aldrich GmbH, Steinheim, Germany) were utilised for goblet cell and mucus formation, and hematoxylin and eosin (H&E) reagent (Merck, Darmstadt, Germany) for the analysis of inflammatory infiltrates. The degree of inflammation and mucous was measured by two separate observers.

**DISCUSSION**
TLR-agonists are being developed to be utilised in conjunction with allergen during SIT in addition to being used alone to treat allergic diseases [9,12–14]. We were therefore curious about the effects that the various TLR-agonists had on the emergence of OVA-specific Th2 responses and if suppression is linked to an increase in allergen-specific Th1 responses. The TLR agonists LTA, poly(I:C), LPS, R848 and CpG-ODN were used in conjunction with OVA/alum to address this subject. The development of airway eosinophilia was found to be considerably and dose-dependently reduced exclusively by the application of LPS and CpG-ODN, an effect previously observed when BCG, heat-killed BCG, or PPD in combination with OVA/alum was used. Nearly 100% of the CpG-ODN dosage was used to achieve the suppressive effect. OVA-specific IgE levels were decreased by all TLR-agonists, but OVA-specific IgG2a levels were enhanced; CpG-ODN and poly(I:C) boosted OVA-specific IgG2a levels by more than 100 times. Additionally, only these two groups had elevated Th1
responses as evidenced by the detection of IFN-γ in the BAL. When applied in conjunction with OVA, LTA, LPS, poly(I:C), or CpG-ODN were found to increase neutrophil counts. Since this was not seen in the mice that were solely given OVA, it is evident that the neutrophilia that was detected was OVA-specific and the result of a changed immunological response to OVA.

More than ten years have passed since the first findings on the possibility of TLR9 agonists to stop the development of allergic responses in mice were released [16]. The great ability of TLR9 to cause a strong immunological deviation from Th2 to Th1 responses was further supported by other findings utilising different allergens, such as birch pollen or house dust mites, as this study also shown. It's unclear exactly how TLR activation impacts asthma, and published findings haven't always been reliable. For instance, Redecke et al. reported that TLR2 ligands exacerbate asthma by biasing the adaptive immune response towards a Th2 phenotype [20]. On the other hand, TLR2 and TLR4 agonists were effective in suppressing allergen-induced pulmonary responses, according to Velasco et al. According to a study by Sel et al., all symptoms of experimental asthma were found to be prevented by activating TLR3 and TLR7 just before to allergen sensitization.

Our investigation revealed an intriguing finding: a large reduction in Th2 responses was not always correlated with the production of strong allergen-specific Th1 responses. The second-strongest Th1-inducing effect was exhibited by poly(I:C), which did not lessen cutaneous anaphylaxis, goblet cell metaplasia in the lung, or serum IgE levels specific to OVA. The fact that only LPS and CpG were able to significantly lower all observed Th2 parameters was another unexpected outcome. This clearly indicates that the effect differed from agonist to agonist and that R848, poly(I:C), and LTA could only influence a portion of the allergen-specific Th2 response. LTA, for instance, decreased levels of IgE, IL-5, and airway eosinophilia, but not IL-4, glandular cell metaplasia, or cutaneous anaphylaxis. On the other hand, R848 decreased IgE levels but not IL-4, IL-5, or airway eosinophilia. Why some factors are impacted while others are not is beyond our understanding. That does, however, obviously depend on the TLR agonist that is employed. All of our data collectively demonstrate that LPS and CpG have the most potent inhibitory impact on the development of several allergen-specific Th2 responses in mice. Our findings encourage more clinical testing of TLR9 and TLR4 agonists, which have already been employed as adjuvants in clinical SIT trials [9, 23]. Curiously, a large reduction in the allergic reaction did not always correlate with a strong induction of allergen-specific Th1 responses, indicating that this indicator of an efficient SIT response could not always result in a reduction in the allergic response.

References

chrane Collaboration method. Allergy, 61, 1162-1172.


