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IgE and IgG epitope mapping by microarray peptide-immunoassay reveals the importance and diversity of the immune response to the IgG3 equine immunoglobulin

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ABSTRACT

The presence of whole horse IgG in therapeutic snake antivenom preparations of high purity is a contamination that can cause IgE-mediated allergic reactions in patients. In this study, the immunodominant IgE and IgG-binding epitopes in horse heavy chain IgG3 were mapped using arrays of overlapping peptides synthesized directly onto activated cellulose membranes. Pooled human sera from patients with and without horse antivenom allergies were used to probe the membrane. We have demonstrated that, for both cases, individuals produce antibodies to epitopes of sequential amino acids of horse heavy chain IgG3, although the signal strength and specificity appear to be distinct between the two groups of patients. A single region was found to contain the dominant allergic IgE epitope. The critical residues involved in the binding of human IgE to the epitope were determined to include four hydrophobic amino acids followed by polar and charged residues that formed a coil structure. This is the first study to describe the specific amino acid sequences involved with the immune recognition of human IgG and IgE to horse antivenom.

1. Introduction

Antivenom is the primary treatment for snake envenoming (Theakston and Warrell, 1991; Lang et al., 2000). These immunobiological compounds contains polyclonal antibodies raised against the one or more venoms and can be obtained in horses, sheep or goats. Intravenous administration of antivenom to patients with snake envenoming prevent or reverse clinical effects by the binding of the antibodies to circulating snake toxins, which neutralizes their activity and promote their elimination (Warrell, 1996). Monovalent antivenoms are raised against a single snake species, while polyvalent antivenoms are raised against more than one specie.

These preparations are normally composed of pepsin-refined immunoglobulin fragments ($F(ab')_2$) that are most

Abbreviations: CBS, 50 mM citrate-buffer saline; hIg, horse immunoglobulin; ELISA, enzyme linked immunosorbent assay; hhcIg, horse heavy chain immunoglobulin; HCS, health control sera; HRP, horseradish peroxidase; NST, not sensitive treatment; ST, sensitive treatment; TBS-CT, tris-buffer saline containing 3% casein and 0.1% Tween 20, pH 7.0; TBS-T, tris-buffer saline, 0.1% Tween 20, pH 7.0; TMB, 3,3′,5,5′-tetramethylbenzidine.

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often prepared from the plasma or serum of horses hyperimmunized with preparations of the particular toxin(s) in question. The appropriate administration of an antivenom preparation can have a profound and rapid impact on the recovery of a victim (Gutiérrez et al., 2006; Isbister et al., 2012). However, immediate hypersensitivity reactions to the foreign proteins in snake antivenoms are the most severe side effects from antivenom treatment. Life threatening reactions can be mediated by patient IgE antibodies (anaphylactic) or non-IgE mechanisms (anaphylactoid) (Chippaux and Goyffon, 1998; Moran et al., 1998; León et al., 2013). Later reactions (5–26 days after administration; serum sickness or type III hypersensitivity) are less severe that involve the immune response of the patient to raise IgG antibodies against the horse proteins (Dart and McNally, 2001; LoVecchio et al., 2003; Vazquez et al., 2013). Published worldwide rates of anaphylactic reactions to antivenom range from 1% (Visser and Chapman, 1978; Christensen, 1981) to 19% (Coetzer and Tilbury, 1982; Isbister et al., 2000) up to a maximum 40% (Wilkinson, 1994). In Brazil, an incidence of about 10–20 cases of anaphylactic reactions for every 20,000 injections has been estimated for the last ten years (Melgarejo, personal communication). Even with these possible side effects, there is good evidence that in cases of severe envenoming the benefits from the treatment with antivenom outweigh the risks of adverse reactions (Warrell, 1996; Chen et al., 2000).

Clinically, anaphylaxis is an exaggerated response of a sensitized patient, someone with circulating IgE antibodies that can react with the proteins administered in the antivenom, which causes the degranulation of mast cells and basophiles. Degranulation leads to the release of histamine, serotonin and other vasoactive substances. An anaphylactic reaction can cause multiple signs and symptoms such as itching, erythema, flushing, urticaria, angioedema, nausea, diarrhea, vomiting and edema of the glottis/larynx, bronchospasm, hypotension, shock and death. Anaphylactoid reactions are clinically similar to anaphylactic reactions, but are not mediated by IgE (Kay, 2001).

Antibodies are proteins produced by B-cells in response to immunogenic substances. Although much is known about the structure-function of this immunological class of proteins, there is limited published evidence about the precise localization of antigenic sites contained within the antibodies themselves (Terness et al., 1995). This is in contrast to their considerable biotechnological and immunotherapeutic importance since the administration of horse immunoglobulin (hIg) preparations in humans carries the inherent risk of inducing an allergic response in a significant number of people (Sutherland and Lovering, 1979; Ellis and Smith, 1988; Demoly et al., 2002; Williams et al., 2007; Morais and Massaldi, 2009). To date, the recognition of hIg by human antibodies and the specific residues involved remain unknown.

Therefore, a detailed molecular characterization of the epitopes in hIg that are bound by human antibodies would greatly contribute to our understanding of the observed adverse reactions. Here, the horse heavy chain of immunoglobulin G3 (hhcIgG3) was examined since it is the major hIg isotype implicated in the snake venom toxins neutralization (Fernandes et al., 2000) and it is the second most prominent antibody in horse serum (Sheoran et al., 2000). In the present study, we have mapped within the entire sequence of hhcIgG3 the epitopes bound by human IgG and IgE along with the critical amino acids within the epitope involved with interactions with human antibodies. The identification of the important amino acids suggests different molecular mechanisms for the binding of IgG and IgE to hhcIgG3.

Localizing antigenic determinants within the polypeptide chains of protein molecules is essential for subsequent investigations of structural and functional characteristics. By identifying and quantifying components in hIg that contribute to immunological discrimination between self and non-self, antivenoms and antitoxins can be improved by new production techniques engineered specifically to minimize activation of a patient's immune response, but maintain efficacy.

2. Material and methods

2.1. Reagents

Amino-PEG_{500}-UC540 cellulose membranes were obtained from Intavis AG Bioanalytical Instruments (Germany). Amino acids for peptide synthesis were purchased from Calbiochem–Novabiochem Corp. (Germany). Acetonitrile, trifluoroacetic acid and other sequence reagents and chemicals were obtained from Merck (Darmstadt, Germany). Rabbit anti-human-IgG and goat anti-human IgE immunoglobulin labeled with alkaline phosphatase were purchased from Abcam plc (USA) and KPL (Kirkegaard & Perri Laboratories, USA), respectively. The immunoperoxidase assay kit for determining the level of IgE in human samples was purchased from Immunology Consultants Lab (Newberg, USA). Super Signal R West Pico and chemiluminescent substrate were acquired from Pierce Biotechnology (Rockford, IL, USA). Bovine serum albumin and Tween 20 were obtained from Sigma–Aldrich.

2.2. Human sera

The pool of human serum that served as the IgG antibodies source was collected from fifteen healthy volunteers who had been treated over a period of 4–12 months with at least one injection of antivenom produced in horses and had no history of hypersensitivity. The source of IgE antibodies was from three selected patients, who had been victims of one or more snake bites and who had presented with a severe anaphylactic reaction when treated with a series of three injections of antivenom (polyvalent antibothropic sera). The concentration of IgE was measured as described below.

The allergic response was confirmed by clinical history and positive response to hIg, and characterized by measuring specific IgE reactivity. A pool consisting of equal volumes of sera from fifteen health individuals who had never received any therapeutically injection products produced in horses were used as a control. Informed consent was obtained from all patients and volunteers. Our study was approved by the Ethics Committee of the Santa Casa de