Veterinary Immunology and Immunopathology 155 (2013) 38–47

Contents lists available at SciVerse ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Research paper

Immunoproteomic characterization of *Ambrosia artemisiifolia* pollen allergens in canine atopic dermatitis

Jana Ognjenovic[a,1], Natalija Milcic-Matic[b,1], Katarina Smiljanic[a,*], Olga Vuckovic[c], Lidija Burazer[c], Nikola Popovic[b], Dragana Stanic-Vucinic[a], Tanja Cirkovic Velickovic[a]

[a] Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia
[b] Department of Dermatology, School of Veterinary Medicine, University of Belgrade, Bulevar Oslobodjenja 18, 11000 Belgrade, Serbia
[c] Institute of Virology, Vaccine and Sera, "Torlak", Vojvode Stepe 458, 11152 Belgrade, Serbia

ARTICLE INFO

Article history:
Received 20 March 2013
Received in revised form 22 May 2013
Accepted 3 June 2013

Keywords:
Amb a 1 allergens
Ambrosia artemisiifolia pollen
Canine atopic dermatitis
Dog
Immunoproteomics
Short ragweed

ABSTRACT

Canine atopic dermatitis (CAD) is an immune system disorder that affects 10–15% of the canine population. Short ragweed (*Ambrosia artemisiifolia*) pollen represents one of the major seasonal sources of allergenic pollen proteins in Europe, particularly in the Pannonian valley of the Balkan region. In Serbia, about 66% of atopic dogs showed a positive intradermal skin test with its pollen extract, which is second to house dust mites. Therefore, characterization of *Ambrosia artemisiifolia* pollen components, in terms of defining major and minor allergens that induce clinically manifested allergic reaction in dogs, is important for valid diagnosis and efficient therapy.

This study has, for the first time, characterized and identified major *Ambrosia artemisiifolia* allergens in CAD, using an immunoproteomic approach. To assess the prevalence of specific IgE in electrophoretically separated ragweed pollen proteins, individual reactivity of sera from dogs with CAD was analyzed and compared to the reactivity of sera from healthy dogs in the non-reducing conditions, which were found optimal for specific canine IgE detection. A specific IgE band (38 kDa) was recognized as the most dominant allergen in CAD, occurring in 81% of positive dog's sera. 2-D immunoblotting followed by a mass spectrometry peptide fingerprint analyses with pooled canine and human atopic sera, revealed that 38 kDa major *Ambrosia atremisiifolia* allergens in CAD were all five isoallergens of the Amb a 1 group (antigen E), including the previously named Amb a 2 (antigen K). In contrast to canine sera, human atopic sera also recognized lower mass allergens such as the β fragment of Amb a 1 and profilins (Amb a 8 variants).

The most prominent ragweed proteins in CAD, represent, as in humans, variants of all five isoallergens of the Amb a 1 group (pectate lyase): Amb a 1.0101 and its natural variant E1XUL2, Amb a 1.0202, 1.0304, 1.0402 and the natural variant of Amb a 1.0501, E1XUM0, as well as the α fragment of pollen allergen Amb a 1.0201.

© 2013 Elsevier B.V. All rights reserved.

Abbreviations: AD, atopic dermatitis; CAD, canine atopic dermatitis; CBB, Coomassie brilliant blue; ID, internal diameter; IDST, intradermal skin test; LTQ, linear ion trap with quadrupole mass filter; MS, mass spectroscopy; nLC–MS/MS, nano-liquid chromatography coupled to tandem mass spectroscopy; PAA, polyacrylamide; PNU, protein nitrogen unit; TTBS, Tris buffered saline with 0.1% Tween 20 (pH 7.4).

* Corresponding author. Tel.: +381 113336661; fax: +381 112184330.
E-mail addresses: katarinas@chem.bg.ac.rs, katicas71@gmail.com (K. Smiljanic).

[1] Equally contributing.

0165-2427/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetimm.2013.06.005

1. Introduction

Canine atopic dermatitis (CAD) is one of the most common skin diseases in dogs (Willemse, 1986; Scott et al., 2001). It is defined as a genetically predisposed inflammatory and pruritic allergic skin disease, with characteristic clinical features associated with IgE antibodies most commonly directed against environmental allergens (Halliwell, 2006). Numerous environmental allergens have been incriminated in the pathogenesis of CAD with house dust mites and pollens being on the top of the list (Hill and DeBoer, 2001). The diagnosis of atopic dermatitis (AD) in dogs relies upon a careful evaluation of history provided by the owner, the presence of appropriate clinical signs, and exclusion of other pruritic skin diseases (Willemse, 1986; Prelaud, 1998; Favrot et al., 2010) and may be confirmed with *in vivo* or *in vitro* tests to demonstrate the presence of allergen-specific IgE.

Some of the clinically important environmental allergens, such as pollens (e.g. trees, grass, weeds) vary with the season, the climate and the geographic region (Sture et al., 1995; Hill and DeBoer, 2001). Pollen of short ragweed (*Ambrosia artemisiifolia*) is the single most seasonal allergen in North America and parts of Europe, affecting up to 36 million human individuals (Wopfner et al., 2009), causing more allergic reactions and related diseases in humans, than all other plants together (Bagarozzi et al., 1998). Short ragweed is becoming a major source of weed pollen allergen in central and southern Europe (Weber, 2005; D'Amato et al., 2007). Recent phyto-geographical and aerobiological studies of the distribution and abundance of short ragweed populations have shown that Serbia is severely affected by this plant and threatened by its airborne pollen (Janjic and Vrbnicanin, 2007; Sikoparija et al., 2009), with pollination periods extending to more than 100 days (Janjic and Vrbnicanin, 2007). The sensitization rate of human subjects allergic to ragweed pollen in the European population is quite high and still increasing; being above 2.5% in all European countries, highest in Hungary (50%), the Netherlands (15.2%) and Germany (14.2%) as shown in a pan-European GALEN study (Jager, 2000; Asero et al., 2006; Burbach et al., 2009).

There is no similar, multiregional study regarding the prevalence of dog's sensitization to ragweed pollen, and this could be due to its wide geographical variation. While some American authors agree that short ragweed is the most significant weed pollen allergen in CAD (Reedy et al., 1997; Scott et al., 2001; Zur et al., 2002), in the Scandinavian countries and Poland, such reports are still absent due to the climate restricted spread of this plant (Taszkun, 2011; Cunze et al., 2013). According to the intradermal skin test (IDST) results from the USA (Scott, 1981; Nesbitt et al., 1984), France (Beco and Fontaine, 1995) and Greece (Saridomichelakis et al., 1999), ragweed pollen causes a positive reaction in 7.7–59% of dogs with AD (Hill and DeBoer, 2001) and 77% of atopic dogs have elevated IgE levels against this pollen, as revealed by ELISA (Kleinbeck, 1994; Hill and DeBoer, 2001). Results from Serbia indicate that ragweed allergens are important in CAD development, since 66% of atopic dogs showed a positive IDST with its pollen extract, right after house dust mites (Milcic-Matic et al., 2010).

Up to date, seven groups of ragweed allergens have been identified in human allergies: the Amb a 1 group or antigen E (pectate lyase) with 5 isoallergen subgroups (http://www.allergen.org/viewallergen.php?aid=32), Amb a 3/Amb a 7 group (plastocyanine), Amb a 4 (defensin-like protein) (Leonard et al., 2010), Amb a 5 homologues, Amb a 6 (lipid transfer protein), Amb a 8 (profilin) and Amb a 9/Amb a 10 (polcalcin/polcalcin-like protein). About 95% of ragweed-sensitized human patients display IgE antibodies to the Amb a 1 major allergen (Adolphson et al., 1978; Løwenstein and Marsh, 1983; Asero et al., 2006; Taramarcaz, 2006). Included in this group is the previously named Amb a 2 allergen group (antigen K), being now assigned as isoallergen Amb a 1.05, which is recognized in about 70% of human patients (Griffith et al., 1991; Rogers et al., 1991). The remaining groups represent minor human allergens.

Progress in immunological characterization of major allergens in human allergies has enabled the development of advanced diagnostic methods and new approaches toward specific immunotherapy of allergies. As with the human atopic population, component-resolved diagnosis and therapy could also result in an increased quality of life in the canine atopic population, by improving the pace and level of recovery. Besides, such an investigation could contribute to a better understanding of the mechanisms responsible for CAD development.

Identification and characterization of ragweed allergens in CAD have not been investigated so far and this is the first study to address this subject. Unlike humans, dogs are very susceptible to parasitic infections, either inherent or acquired, and the defense against numerous parasites accounts for the presence of high serum concentrations of IgE antibodies in healthy animals (Meeusen, 1999; Ledin et al., 2006). As a result, detection of very low concentrations of specific IgE antibodies to allergens is very complex and difficult in terms of methodology. With the new methodological approach using immunoproteomic research with a crude ragweed pollen extract, the chapter of *A. artemisiifolia* allergen characterization in CAD has been opened. We present the major ragweed allergens against which specific-IgE antibodies were detected in the sera of dogs with AD by the means of 2-D PAGE electrophoresis, immunoblotting and nano-liquid chromatography coupled with tandem mass spectroscopy (nLC–MS/MS).

2. Materials and methods

2.1. Study population

Dogs with AD were selected from the Dermatology clinic at the Faculty of Veterinary Medicine, Belgrade University, Serbia, during the period of the short ragweed pollination season. Diagnosis was based on a combination of a compatible history and clinical signs, and exclusion of other pruritic dermatoses (Willemse, 1986; Prelaud, 1998; Favrot et al., 2010). Coat brushings, skin scrapings and trial therapy were used to eliminate ectoparasites. All dogs underwent a 6-week, commercial hypoallergenic diet to eliminate

Download English Version:

https://daneshyari.com/en/article/5796880

Download Persian Version:

https://daneshyari.com/article/5796880

Daneshyari.com