

Hyaluronidase and Hyaluronan in Insect Venom Allergy

Te Piao King Knut M. Wittkowski

The Rockefeller University, New York, N.Y., USA

Key Words

Insect • Bee • Vespid • Venom • Hyaluronidase • Hyaluronan and allergy

Abstract

Background: Insect venoms contain an allergen hyaluronidase that catalyzes the hydrolysis of hyaluronan (HA), a polymer of disaccharide GlcUA-GlcNAc in skin. HAs depending on their size have variable function in inflammation and immunity. This paper reports on whether hyaluronidase, HA polymers and oligomers can promote antibody response in mice. **Methods:** HA oligomers (8- to 50-mer; 3–20 kDa) were obtained by bee venom hyaluronidase digestion of HA polymers (750- to 5,000-mer; 300–2,000 kDa). Antibody responses in mice were compared following 3 biweekly subcutaneous injection of ovalbumin (OVA) with or without test adjuvant. **Results:** OVA-specific IgG1 levels were approximately 2 times higher in BALB/c and C3H/HeJ mice receiving OVA and HA oligomer or polymer than those treated with OVA alone, and no increase in total IgE level was observed. In C57Bl/6 mice, observed increases in IgG1 and IgE were 3.5- and 1.7-fold, respectively, for the oligomer and 16- and 5-fold ($p < 0.05$), respectively, for the polymer. **Conclusion:** Hyaluronidase by its action on HA in skin can function indirectly

as adjuvant to promote IgE and IgG1 response in mice. Insect venoms also have cytolytic peptides and phospholipases with inflammatory roles. These activities found in mice may contribute to venom allergenicity in susceptible people.

Copyright © 2011 S. Karger AG, Basel

Introduction

Most insect allergies are caused by bee and vespid stings; vespids include hornets, yellow jackets and paper wasps. Bee and vespid venoms each contain several protein allergens. One allergen common to these venoms is hyaluronidase. Hyaluronidases from these insects have varying degrees of antigenic identity as they have similar sequences and structures [1, 2]. They have identical enzymatic specificity of hydrolysis of a tissue constituent hyaluronan (HA), previously known as hyaluronic acid. HA is a polymer of $>10^4$ -mer of the disaccharide GlcUA-GlcNAc with molecular weight of $>4 \times 10^3$ kDa. Its concentration in human dermis is about 0.2 mg/ml [3].

High molecular weight HA polymers are ubiquitously distributed in tissues and are believed to be anti-inflammatory, while low molecular weight HA polymers ($<10^4$ -mer) released on tissue injury are pro-inflammatory [4,

5]. Low molecular weight HA polymer bind Toll-like receptor (TLR) 2 of macrophages and dendritic cells, and functions as adjuvant for antigen-specific T cell response in mice but not high molecular weight HA polymer [6]. High and low molecular weight HAs have been implicated in their roles to regulate T regulatory cells [7]. HA polymer can activate B cells on binding to CD44 receptor [8]. HA oligomers also have active roles and one example is HA 4- to 6-mer binding to TLR 4 of dendritic cells to stimulate their maturation and production of cytokines [9]. In addition to TLR and CD44, another cellular HA receptor of interest in immunity is LYVE-1 in lymphatic endothelium [10]. Studies with CD44 receptor show a size-dependent increasing affinity for HA oligomers reaching plateau with >10-mer [11] and the binding is irreversible for HA polymers >80-mer [12].

The published data suggest that low molecular weight HA polymers and oligomers in skin released on insect sting may function as adjuvant to promote venom allergenicity. In this paper the size distribution pattern of HA oligomers released on venom hyaluronidase hydrolysis and the adjuvant action of HA oligomers (10- to 100-mer) and polymers (750- to 5,000-mer) for antibody response in mice will be reported.

Materials and Methods

Materials

HA polymers sodium salt from bovine vitreous, rooster comb and *Streptococcus equi* sp. as well as HA polymer potassium salt from human umbilical cord were obtained from Sigma-Aldrich with molecular weights of 300, 1,630, 2,000 and 750 kDa, respectively; corresponding to 750-, 4,100-, 5,000- and 1,900-mer, respectively. The molecular weights are average values supplied by the vendors and the samples are polydisperse in their size.

Hyaluronidases from bee (*Apis mellifera*) and yellow jacket (*Vespula vulgaris*) venoms were isolated as described [13, 14] and that from bovine testes (type VIII) was obtained from Sigma-Aldrich. Hyaluronidase activity was assayed by its release of the reducing group of GlcNAc [15] from 2 mg/ml of HA sodium salt from rooster comb at pH 5. The venom enzymes each released 880 nmol/min of GlcNAc/mg and the testes enzyme 3 nmol/min/mg at room temperature.

Size Distribution and Isolation of HA Oligomers on Venom Hyaluronidase Hydrolysis

A solution of 8–10 mg/ml HA polymer in pH 5 buffer of 0.1 M NaAc + 0.083 M NaCl, or pH 7 buffer of 7.5 mM sodium phosphate + 0.15 M NaCl, was digested with 3 µg/ml venom hyaluronidase at ambient temperature for 1–4 days. Hyaluronidase was inactivated at end of digestion by heat at 100° for 10 min. Digests were analyzed by gel electrophoresis and gel filtration.

Electrophoresis was made with 5–10 µl of digest in 15% polyacrylamide gel (0.3 × 10 × 10 cm) at 240 volts for 50–80 min

and gel was stained with 0.2% Alcian Blue dye. HA oligomer sizes were estimated by assigning the band co-migrating with the tracking dye bromophenol blue to be 8-mer [16].

Gel filtration of 50 mg HA digests was made on a 0.9 × 100 cm column of Sephadex G50 or G100. The column was eluted with 0.1 M NH₄CO₃ at 12 ml/h. Desired fractions were lyophilized and dissolved in water. Concentration of HA oligomers was estimated by absorbance at 206 nm using a value of 4 as 1 mg/ml and determined by GlcUA content [17].

Comparing Adjuvant Action of HA Oligomer/Polymer or Alum in Mice

BALB/c, C3H/HeJ and C57BL/6 mice were from Jackson Laboratory or Charles River. Groups of 3 mice (20–22 g) were each immunized with 10 µg ovalbumin (OVA) and 0.5 mg HA oligomer/polymer or 0.3 mg alum in 0.1 ml phosphate-buffered saline by subcutaneous route on weeks 0, 2 and 4. Their sera were collected on weeks 1, 3 and 5. These studies were done under conditions given in the Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animals Resources, National Research Council, and published by the National Academy Press (revised 1996).

OVA-specific IgG1 was detected by ELISA in wells coated with 0.1 mg/ml OVA, followed by 1 µg/ml biotinylated goat anti-mouse IgG1 (Southern Biotechnology) and 1 µg/ml avidin-peroxidase conjugate (Sigma-Aldrich). Total IgE was detected in wells coated with 10 µg/ml 6HD5 antibody specific for Fc region of mouse IgE [18], followed by 1.5 µg/ml biotinylated rabbit anti-mouse IgE and 1 µg/ml avidin-peroxidase conjugate. Bound avidin-peroxidase was detected as described [19]. Antibody concentration was estimated from standard curves of melittin-specific monoclonal IgG1 and IgE determined on melittin and 6HD5 coated wells, respectively.

Statistical analyses of data were made using the Student t test of two samples assuming unequal variances in Microsoft Excel. p values after Bonferroni correction for multiple sample comparison were made using the Sidak equation and are designated as p_c (<http://www.utdallas.edu/~herve/Abdi-Bonferroni2007-pretty.pdf>).

Results

Size Distribution of HA Oligomers on Hyaluronidase Hydrolysis

After 3-day digestion of *Streptococcus* HA with bee enzyme at pH 7 the major products are oligomers of large size range as indicated by the relative heights of early and late eluting bands on Sephadex G100 gel filtration in figure 1a, and the 1-day digest at pH 5 contains mainly the late band of small HA oligomers. Similar results were observed with rooster comb HA when digested with bee or yellow jacket venom, or testicular hyaluronidase, at equivalent enzyme and substrate concentrations (results not shown).

Oligomers used for adjuvant tests were obtained from G50 gel filtration of pH 7 digests after 4 days (fig. 1b). The

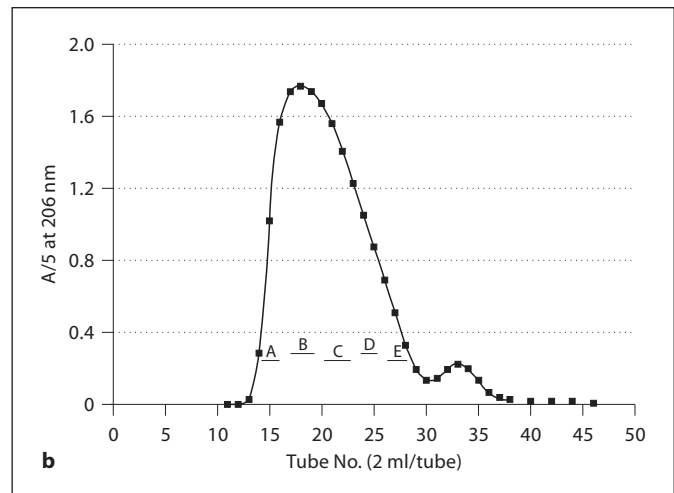
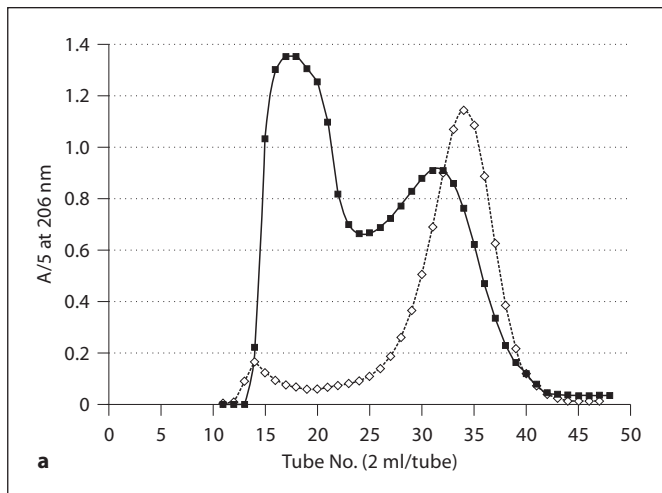


Fig. 1. a Sephadex G100 gel filtration of bee venom hyaluronidase digests of HA. Solid curve is for pH 7 digest after 3 days and dashed curve is pH 5 after 1 day. **b** Sephadex G50 gel filtration of 4-day pH 7 digest of HA by bee venom hyaluronidase. A–E: see figure 2.

pooled fractions A–E from figure 1b are found to contain oligomers of overlapping sizes 20- to 80-, 14- to 50-, 10- to 30-, 8- to 18- and 6- to 12-mers, respectively, on polyacrylamide gel electrophoresis analysis (fig. 2). HA oligomers are separated into bands of decreasing sizes, each differing by one disaccharide unit and the band co-migrating with the tracking dye bromophenol blue is taken to be 8-mer. Oligomers are visualized by dye staining with the exception of oligomers of <6-mer which are not detectable by dye staining. HA oligomers in the size range of 6- to 40-mer are well resolved as distinct bands under the conditions used, but oligomers of >40-mer are not.

The fractions of 14- to 50-, 10- to 30- and 6- to 12-mers were also characterized by their molar ratio of GlcUA to GlcNAc with terminal reducing group, and they were 31, 25 and 7.0, respectively.

Testing Adjuvant Action of HA Oligomers/Polymers and Hyaluronidase

Groups of 3 mice of BALB/c, C3H/HeJ or C57Bl/6 strains were immunized with OVA and test adjuvant, and their sera at week 5 after 3 biweekly subcutaneous injections were measured for antibodies. Their OVA-specific IgG1 and total IgE are given in figure 3 as 5 separate sets of none, alum, oligomer, polymer and hyaluronidase; each set has 3–12 groups of mice. HA oligomers 14- to 50- and 10- to 30-mer were tested as separate groups and their results shown in different symbols are presented together as that of oligomer set in figure 3. Similarly, the

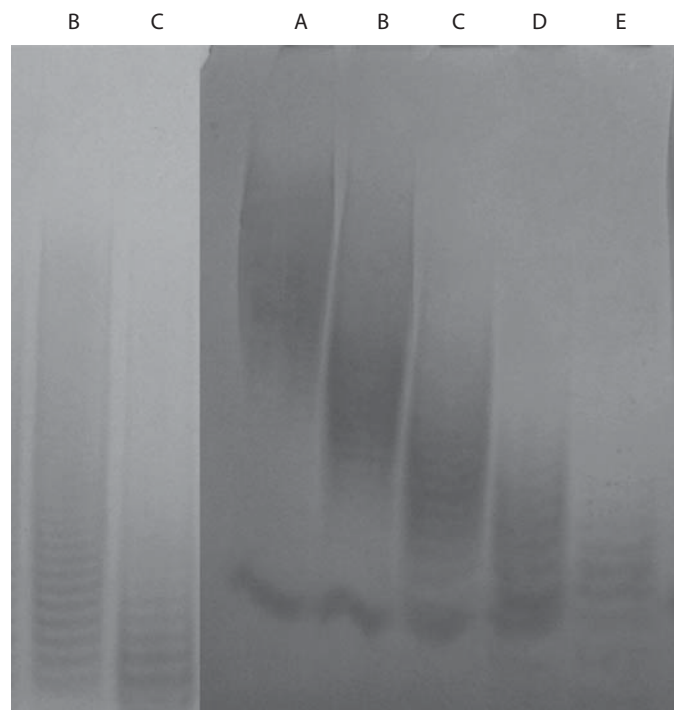


Fig. 2. Polyacrylamide gel electrophoresis pattern of HA fractions from figure 1b. Fractions were separated for 46 and 75 min at 120 v in the right and left hand patterns, respectively.

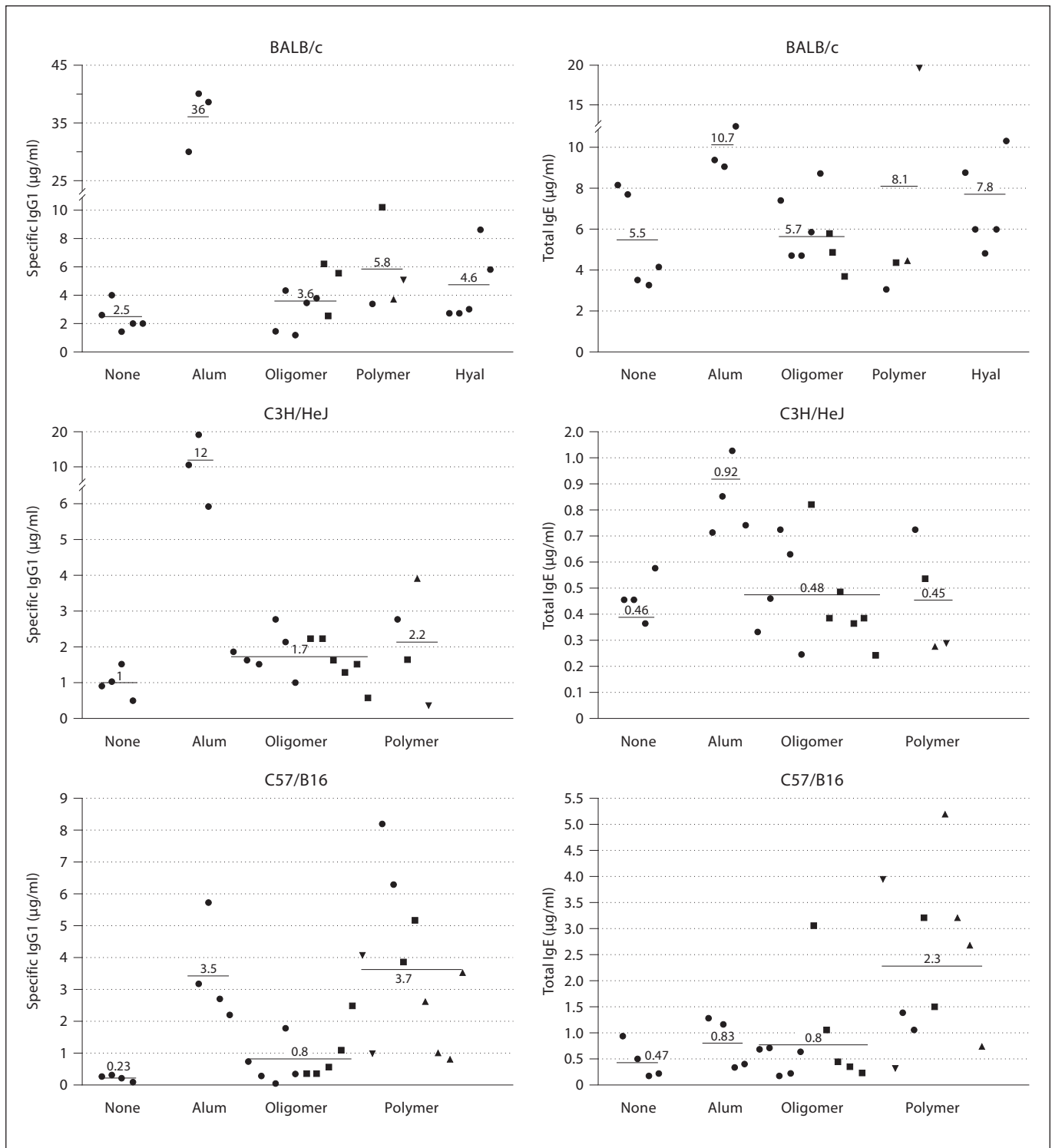


Fig. 3. Specific IgG1 and total IgE responses in BALB/c, C3H/HeJ and C57/Bl6 mice on immunization with OVA and test adjuvant. Each symbol in the figure represents one group of 3 mice. For HA oligomer, the circle and square symbols represent data from 10- to 30- and 14- to 50-mer, respectively. For HA polymer, the circle, square, triangle and inverted triangle symbols represent 750-, 1,900-, 4,100- and 5,000-mer, respectively. Average antibody value of each set is given above the bar; these data and their relative data as well as p and p_c values are summarized in table 1.

Table 1. Summary of average antibody levels of different sets from figure 3

Adjuvant	Ab	Average IgG1/IgE, µg/ml (p and p _c values), and relative IgG1/IgE levels		
		BALB/c	C3H/HeJ	C57Bl/6
None	IgG1	2.5; 1	1.0; 1	0.23; 1
	IgE	5.5; 1	0.46; 1	0.47; 1
Alum	IgG1	36 (p = 0.008, p _c = 0.031); 14	12.0 (p = 0.109, p _c = 0.293); 12	3.5 (p = 0.026, p _c = 0.076); 15
	IgE	10.7 (p = 0.042, p _c = 0.158); 1.9	0.92 (p = 0.095, p _c = 0.259); 2.0	0.83 (p = 0.272, p _c = 0.614); 1.8
Oligomer	IgG1	3.6 (p = 0.170, p _c = 0.525); 1.4	1.7 (p = 0.026, p _c = 0.076); 1.7	0.80 (p = 0.050, p _c = 0.143); 3.5
	IgE	5.7 (p = 0.860, p _c = 1.000); 1.0	0.48 (p = 0.781, p _c = 0.989); 2.0	0.80 (p = 0.311, p _c = 0.673); 1.7
Polymer	IgG1	5.8 (p = 0.169, p _c = 0.523); 2.3	2.2 (p = 0.227, p _c = 0.538); 2.2	3.7 (p = 0.002, p _c = 0.006); 16
	IgE	8.1 (p = 0.540, p _c = 0.955); 1.5	0.45 (p = 0.935, p _c = 1.000); 1	2.3 (p = 0.005, p _c = 0.015); 4.9
Hyal	IgG1	4.6 (p = 0.141, p _c = 0.456); 1.8	not done	not done
	IgE	7.8 (p = 0.147, p _c = 0.470); 1.4		

p values after Bonferroni correction are designated as p_c values. p_c = 1 - (1 - p)ⁿ with n = 4 for BALB/c and n = 3 otherwise.

results of 4 HA polymers of 750- to 5,000-mer are treated as one polymer set. Oligomer 6- to 12-mer did not show significant adjuvant activity (results not shown). Hyaluronidase was tested only in BALB/c mice.

The average antibody data for each set in figure 3, their p values before and after Bonferroni correction and antibody levels relative to the control are summarized in table 1. Compared to the control set, alum sets show IgG1 and IgE level increases of 12–15 and 1.8–2 times (p = 0.008–0.109 and 0.042–0.272), respectively, in the 3 mice strains. HA oligomer or polymer sets show IgG1 level increases of 1.4–2.3 times (p = 0.03–0.23) in BALB/c and C3H/HeJ mice, and no significant increase in total IgE level was found. Higher IgG1 and IgE increases of 3.5- and 1.7-fold (p = 0.05 and 0.31), respectively, were observed for the oligomer set in C57Bl/6 mice, but more prominent for the polymer set with IgG1 and IgE 16 and 5 times (p = 0.002 and 0.005), respectively, higher. These results were obtained with 0.5 mg of HA oligomers or polymers as adjuvant. Limited tests with one tenth the dose were made with similar findings (results not given).

Of the three mice strains, BALB/c and C57BL/6 are high and low responders to OVA, respectively, and C3H/HeJ is a moderate responder. C3H/HeJ mice are known to be insensitive to lipopolysaccharide. Thus the adjuvant activity of HA oligomers or polymers observed in C3H/HeJ mice or the other two mice strains is unlikely due to possible contamination with lipopolysaccharide [20].

Bee hyaluronidase was tested for adjuvant activity of OVA response in BALB/c mice. As shown in figure 3,

their specific IgG1 and IgE levels were increased by 1.8- and 1.4-fold (p = 0.14 and 0.15), respectively. The dose of hyaluronidase was 0.5 µg similar to those of a single bee sting. A single bee sting is estimated to contain about 60 µg of peptides and proteins [21], and hyaluronidase represents 1% of venom peptides and proteins [22].

Discussion

Bee venom hyaluronidase digestion of HA at pH 7 yielded larger oligomers than that at pH 5. This pattern of product distribution for venom hyaluronidase is the same as testicular hyaluronidase, which is known to have both glycosidase and transglycosidase activities. The expected limit product of HA from glycosidase activity is a tetrasaccharide as the substrate-binding pocket of hyaluronidase can accommodate a pentasaccharide [1]. Because of its transglycosidase activity, the product is a mixture of oligomers of different sizes. The size difference of HA oligomers on digestion at pH 5 and 7 is due to the fact that transglycosidase activity is favored at pH 7 rather than pH 5, while the opposite is the case for glycosidase activity [23].

HA oligomers and polymers are found to promote IgG1 and IgE response in BALB/c, C3H/HeJ and C57Bl/6 mice. However, only the data for HA polymers in C57BL/6 mice are statistically significant. HA polymers of 750- to 5,000-mer appear to be more active than oligomers of 10- to 50-mer, and HA of 6- to 12-mer appear to be inactive. These differences are probably related to size-dependent

affinity of HA oligomers and irreversible binding of HA polymer for their receptor as shown for CD44 [11, 12]. HA polymer is particularly effective as an adjuvant in the low responder C57Bl/6 mice as the increase in IgG1 and IgE levels is as high as that observed with the control adjuvant alum.

HA polymers in skin normally do not stimulate inflammatory or immune responses [5]. This may be related to the presence of specific binding proteins so that HA polymers are organized into complex structures [24]. Tissue injury caused by HA polymer injected into the skin with subsequent formation of low molecular weight polymers and oligomers by hyaluronidase present in tissue may be an explanation of adjuvant activity of HA polymers. This hypothesis is supported by our observation of adjuvant activity of venom hyaluronidase in BALB/c mice. HA polymers have various medical uses and inflammatory responses have been observed with some preparations in patients, with reports suggesting that this may be caused by immune response to contaminant proteins [25, 26]. These findings also support possible adjuvant action of HA polymers in humans.

Bee and vespid venoms each contain other allergens in addition to hyaluronidase; two of them are phospholipase and antigen 5 (a protease of unknown specificity) [27]. T cell epitopes of bee venom phospholipase and vespid venom antigen 5 have been studied and no difference has been observed in allergic and nonallergic people [28, 29]. Structural studies indicate the B cell epitopes of bee venom phospholipase [30] and hyaluronidase [31] as well as vespid antigen 5 [32] are located on the protein surface with no unusual features.

Bee phospholipase can induce IL-4 release from murine mast cells and induce IgE response on low-dose im-

munization in mice, and these activities are linked with its enzymatic activity [33]. In addition to proteins, insect venoms contain cytolytic peptides, mellitin in bees and mastoparan in vespids. The cytolytic peptides and phospholipase can induce inflammatory response of murine macrophages with release of prostaglandin E2 [34].

Studies are being made to understand what makes an allergen [35, 36]. Is it the structure and function of the allergenic protein, and/or other chemical/biological factors in the environment or the host? Analysis of structure and function of over 900 allergens from various sources shows that allergens belong to a small number of protein families with limited molecular functions. One sixth of the known allergens have hydrolase activity of proteins, polysaccharides or lipids [37]. These hydrolase activities are present in insect venom as antigen 5, hyaluronidase and phospholipase. As noted above, venom allergens do not appear to have antigenic properties different from proteins of other sources. It is more likely that venom allergenicity is due to the combined function of hyaluronidase, phospholipase and cytolytic peptides to activate accessory cells to secrete cytokines that trigger the development of T helper and/or regulatory cells and they in turn regulate the development of IgE- or IgG-producing B cells in susceptible people.

Acknowledgements

The authors would like to thank Mr. Craig Hunter of the Comparative Bioscience Center of Rockefeller University for animal work. Research was supported by Rockefeller University. The authors have no financial relationship with any commercial ventures.

References

- Markovic-Housley Z, Miglierini G, Soldato-va L, Rizkallah PJ, Muller U, Schirmer T: Crystal structure of hyaluronidase, a major allergen of bee venom. *Structure* 2000;8: 1025–1035.
- Skov LK, Seppala U, Coen JJ, Crickmore N, King TP, Monsalve R, et al: Structure of recombinant Ves v 2 at 2.0 Angstrom resolution: structural analysis of an allergenic hyaluronidase from wasp venom. *Acta Crystallogr D* 2006;62:595–604.
- Laurent TC, Fraser JRE: Hyaluronan. *FASB J* 1992;6:2397–2404.
- Stern R, Asari A, Sugahara K: Hyaluronan fragments: an information-rich system. *Eur J Cell Biol* 2006;85:699–715.
- Jiang D, Liang J, Noble PW: Hyaluronan in tissue injury and repair. *Ann Rev Cell Dev Biol* 2007;23:435–461.
- Scheibner KA, Lutz MA, Boodoo S, Fenton MJ, Powell JD, Horton MR: Hyaluronan fragments act as an endogenous danger signal by engaging TLR2. *J Immunol* 2006;177: 1272–1281.
- Bollyky PL, Falk BA, Wu RP, Buckner JH, Wight TN, Nepom GT: Intact extracellular matrix and the maintenance of immune tolerance: high molecular weight hyaluronan promotes persistence of induced CD4+ CD25+ regulatory T cells. *J Leuko Biol* 2009; 86:567–572.
- Rafi A, Nagarkatti M, Nagarkatti P: Hyaluronate-CD44 interactions can induce murine B-cell activation. *Blood* 1997;89:2901–2908.
- Termeer C, Benedix F, Sleeman J, Fieber C, Voith U, Ahrens T, et al: Oligosaccharides of hyaluronan activate dendritic cells via Toll-like receptor 4. *J Exp Med* 2002;195:99–111.

- 10 Jackson DG: Immunological functions of hyaluronan and its receptors on the lymphatics. *Immunol Rev* 2009;230:216–231.
- 11 Lesley J, Hascall V, Tammi M, Hyman R: Hyaluronan binding by cell surface CD44. *J Biol Chem* 2000;275:26967–26975.
- 12 Wolny P, Banerji S, Gounou C, Brisson A, Day A, Jackson D, et al: Analysis of CD44-hyaluronan interactions in an artificial membrane system: insights into the distinct binding properties of high and low molecular weight hyaluronan. *J Biol Chem* 2010;285:30170–30180.
- 13 King TP, Sobotka AK, Kochoumian L, Lichtenstein LM: Allergens of honeybee venom. *Arch Biochem Biophys* 1976;172:661–671.
- 14 King TP, Lu G, Gonzalez M, Qian NF, Soldatova L: Yellow jacket venom allergens, hyaluronidase and phospholipase: sequence similarity and antigenic cross reactivity with their hornet and wasp homologs and possible implications for clinical allergy. *J Allergy Clin Immunol* 1996;98:588–600.
- 15 Reissig JL, Strominger JL, Leloir LF: A modified colorimetric method for the estimation of N-acetylamino sugars. *J Biol Chem* 1955;217:959–966.
- 16 Turner R, Cowan M: Cationic dye binding by hyaluronate fragments: dependence on hyaluronate chain length. *Arch Biochem Biophys* 1985;237:253–260.
- 17 Bitter T, Muir H: A modified uronic acid carbazole reaction. *Anal Biochem* 1962;4:330–334.
- 18 Hirano T, Yamakawa N, Miyajima H, Maeda K, Takai S, Ueda A, et al: An improved method for the detection of IgE antibody of defined specificity by ELISA using rat monoclonal anti-IgE antibody. *J Immunol Meth* 1989;119:145–150.
- 19 King TP, Kochoumian L, Joslyn A: Melittin-specific monoclonal and polyclonal IgE and IgG1 antibodies from mice. *J Immunol* 1984;133:2668–2673.
- 20 Eisenbarth S, Piggott D, Huleatt J, Visintin I, Henrick C, Bottomly K: Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002;196:1645–1651.
- 21 Hoffman DR, Jacobson RS: Allergens in hymenoptera venom. XIII. How much protein is in a sting? *Ann Allergy* 1984;52:276–284.
- 22 Habermann E: Bee and wasp venoms. *Science* 1972;177:314–322.
- 23 Saitoh HTK, Majima M, Nakamura T, Matsuki A, Kasai M, Narita H, Endo M: Enzymic reconstruction of glycosaminoglycan oligosaccharide chains using the transglycosylation reaction of bovine testicular hyaluronidase. *J Biol Chem* 1995;270:3741–3747.
- 24 Day AJ, Motte CAD: Hyaluronan cross-linking: a protective mechanism in inflammation? *Trends Immunol* 2005;26:637–643.
- 25 Ottaviani RA, Wooley P, Song Z, Markel DC: Inflammatory and immunological responses to hyaluronan preparations. *J Bone Joint Surg Am* 2007;89:148–157.
- 26 Sasaki M, Miyazaki T, Nakamura T, Takahashi T, Miyauchi S, Iwata H: Immunogenicity of Hylan G-F 20 in guinea pigs and mice. *J Rheumatol* 2004;31:943–950.
- 27 King TP, Monsalve RI: Allergens from stinging insects: ants, bees and vespids; in Kay AB, Kaplan AP, Bousquet J, Holt P (eds): *Allergy and Allergic Diseases*, ed 2. London, Blackwell Publishing, 2007.
- 28 Carballido JM, Carballido-Perrig N, Kagi MK, Meloen MH, Wuthrich B, Heusser CH, et al: T cell epitope specificity in human allergic and non-allergic subjects to bee venom phospholipase A2. *J Immunol* 1993;150:3582–3591.
- 29 Bohle B, Zwolfer B, Fischer G, Seppala U, Kinaciyan T, Bolwig C, et al: Characterization of the human T cell response to antigen 5 from *Vespula vulgaris* (Ves v 5). *Clin Exp Allergy* 2005;35:367–373.
- 30 Dudler T, Schneider T, Annand RR, Gelb MH, Suter M: Antigenic surface of bee venom allergen phospholipase A2. *J Immunol* 1994;152:5514–5522.
- 31 Padavattan S, Schirmer T, Schmidt M, Akdis C, Valenta R, Mittermann I, et al: Identification of a B-cell epitope of hyaluronidase, a major bee venom allergen, from its crystal structure in complex with a specific Fab. *J Mol Biol* 2007;368:742–752.
- 32 Henriksen A, King TP, Mirza O, Monsalve RI, Meno K, Ipsen H, et al: Major venom allergen of yellow jackets, Ves v 5: structural characterization of a pathogenesis-related protein superfamily. *Proteins* 2001;45:438–448.
- 33 Dudler T, Machado DC, Kolbe L, Annand RR, Rhodes N, Gelb MH, et al: A link between catalytic activity, IgE-independent mast cell activation, and allergenicity of bee venom phospholipase A2. *J Immunol* 1995;155:2605–2613.
- 34 King TP, Sui YJ, Wittkowski KM: Inflammatory role of two venom components of yellow jackets (*Vespula vulgaris*): a mast cell degranulating peptide mastoparan and phospholipase A1. *Int Arch Allergy Immunol* 2003;131:25–32.
- 35 Karp CL: Guilt in intimate association: what makes an allergen an allergen? *J Allergy Clin Immunol* 2010;125.
- 36 Traidl-Hoffmann C, Jakob T, Behrendt H: Determinants of allergenicity. *J Allergy Clin Immunol* 2009;123:558–566.
- 37 Radauer C, Bublin M, Wagner S, Mari A, Breiteneder H: Allergens are distributed into few protein families and possess a restricted number of biochemical functions. *J Allergy Clin Immunol* 2008;121:847–852.