Hymenoptera stings can cause severe anaphylaxis in untreated venom-allergic patients. A correct diagnosis regarding the relevant species for immunotherapy is often hampered by clinically irrelevant cross-reactivity. In vespid venom allergy, cross-reactivity between venoms of different species can be a diagnostic challenge. To address immunological IgE cross-reactivity on molecular level, seven recombinant antigens 5 of the most important Vespoidea groups were assessed by different diagnostic setups. The antigens 5 of yellow jackets, hornets, European and American paper wasps, fire ants, white-faced hornets, and Polybia wasps were recombinantly produced in insect cells, immunologically and structurally characterized, and their sIgE reactivity assessed by ImmunoCAP, ELISA, cross-inhibition, and basophil activation test (BAT) in patients with yellow jacket or Polistes venom allergy of two European geographical areas. All recombinant allergens were correctly folded and structural models and patient reactivity profiles suggested the presence of conserved and unique B-cell epitopes. All antigens 5 showed extensive cross-reactivity in sIgE analyses, inhibition assays, and BAT. This cross-reactivity was more pronounced in ImmunoCAP measurements with
venom extracts than in sIgE analyses with recombinant antigens 5. Dose-response curves with the allergens in BAT allowed a differentiated individual dissection of relevant sensitization. Due to extensive cross-reactivity in various diagnostic settings, antigens 5 are inappropriate markers for differential sIgE diagnostics in vespid venom allergy. However, the newly available antigens 5 from further vespid species and the combination of recombinant allergen-based sIgE measurements with BAT represents a practicable way to diagnose clinically relevant sensitization in vespid venom allergy.