

# IgE antibodies in tick bite-induced anaphylaxis

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The western black-legged tick, *Ixodes pacificus*, parasitizes reptiles, birds, and mammals throughout western North America.<sup>1</sup> In California, it is the primary vector of *Borrelia burgdorferi*, the spirochete causing Lyme disease, and may also carry *Francisella tularensis* and a rickettsia of the spotted-fever group.<sup>2-4</sup> Toxic local reactions are common, but systemic IgE-mediated reactions have never been documented.<sup>5</sup> With the exception of the Australian paralysis tick, *I. holocyclus*, allergic reactions to tick bites have not been reported.<sup>6</sup> We recently described a patient who had recurrent anaphylaxis after bites with *I. pacificus*.<sup>7</sup> The study in this article revealed that his serum contained IgG and IgE antibodies to tick extract and that his basophils released histamine on challenge with the extract.

Tick extract was prepared from unfed female *I. pacificus* by pestling in a pool of liquid nitrogen. The powder was suspended in a small volume of 0.1 mol/L of carbonate buffer, pH 9.6, and vortexed at 4° C for 48 hours. After centrifugation at 15,000 g for 15 minutes, the supernatant was aspirated and stored at -80° C. The extract was diluted in phosphate-buffered saline to a concentration of 40 µg/ml. For SDS-PAGE, the extract was dialyzed for 48 hours at 4° C against PAGE-running buffer (0.025 mol/L of Tris, 0.192 mol/L of glycine, and 0.1% of SDS).

SDS-PAGE, ELISA, and an immunoblotting assay for tick-specific IgE and IgG were performed as previously described.<sup>8</sup> Basophil-enriched blood leukocyte HR was performed by monoclonal ELISA, as

## Abbreviations used

SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis  
HR: Histamine release

previously described.<sup>9</sup> In our study, the HR assay has a sensitivity of ~1 ng/ml and is reproducible in the linear 1 to 50 ng/ml range, with an intra-assay coefficient of variation of 10% to 13% and mean inter-assay coefficient of variation of 18%.<sup>10</sup>

The patient's total serum IgE was 109 IU/ml, and a RAST screen for IgE antibodies to common allergens was negative. Serum-IgE antibody against tick extract was positive by ELISA on three occasions (net optical density at 492 nm of 0.194, 0.403, and 0.404) with no reactivity observed in pooled control sera from 12 nonatopic control subjects with no history of adverse reaction to tick bites. His serum also contained high-titered IgG to tick extract, with ELISA reactivity (net optical density >0.3) at serum dilutions >1:5,000, whereas control sera contained no detectable IgG antibody. Preincubation of his serum with tick extract, but not control allergen (*Lol p I*, National Institute of Allergy and Infectious Diseases, Bethesda, Md.), caused a dose-dependent inhibition of ELISA binding. Preincubation of sera from two ryegrass sensitive patients with *Lol p I* inhibited ELISA reactivity to this allergen, whereas it had no effect on background binding to tick extract.

SDS-PAGE of tick extract yielded multiple polypeptides on India ink staining with heavy clustering between 14 to 45 kd.<sup>7</sup> The patient's serum-IgE antibodies reacted with seven allergens of 23 to >110 kd, with a 107 kd allergen revealing the most intense binding. The patient's serum-IgG antibodies reacted with 12 polypeptides between 16 to 40 kd. Serum-dependent nonspecific binding to several polypeptides was observed on immunoblotting, as previously reported for sera from *I. holocyclus*-allergic subjects,<sup>11</sup>

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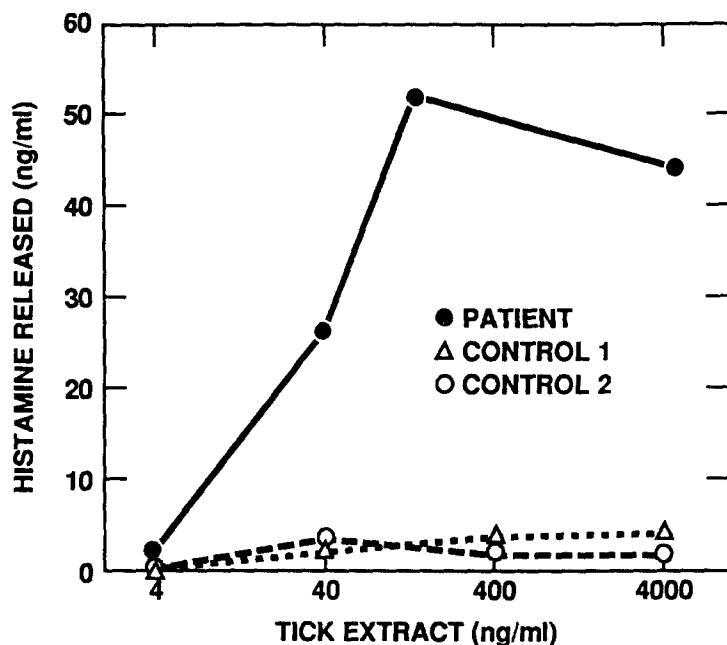


FIG. 1. Basophil histamine release induced by whole-body tick extract in the tick-allergic patient and in two ryegrass-allergic control subjects.

but this nonspecific binding did not account for most of the specificities in the patient's serum. Shared IgG/IgE reactivities were present at 23 and 35 kd.

Basophils were stimulated with tick extract and control allergens. The patient's basophils released histamine in a dose-dependent fashion in response to stimulation with tick extract with  $>20$  ng/ml, or  $>20\%$  total, of histamine released by  $\geq 40$  ng/ml of tick protein (Fig. 1). No histamine was released by stimulation of control basophils at tick concentrations up to 40  $\mu$ g/ml. The tick-allergic patient's basophils did not release histamine on stimulation with *Lol p I*, whereas basophils from ryegrass-allergic control subjects (nonresponsive to tick extract at 40  $\mu$ g/ml) responded with dose-dependent HR to *Lol p I* between 10 ng and 10  $\mu$ g/ml. Basophils from a birch-allergic subject, also nonresponsive to tick, responded to birch (Hollister-Stier Laboratories, Spokane, Wash.) between  $10^{-7}$  and  $10^{-4}$  dilutions.

Anaphylactic reactions to ticks and tick-specific IgE have previously been reported only in Australia after the bite of the paralysis tick, *I. holocyclus*.<sup>6</sup> Several allergens of 23 to 55 kd have been identified in extracts from *I. holocyclus*.<sup>11</sup> Our extract from the American tick, *I. pacificus*, yielded a unique allergen of 107 kd not present in *I. holocyclus* whole-body or salivary extracts, although several other allergens may be shared between the two species.

Our patient has been able to avoid further systemic reactions to tick bites by killing the ticks in situ with kerosene or pyrethrin-containing tick repellent and allowing them to detach spontaneously within several hours. It is generally recommended, however, that attached ticks be removed immediately with tweezers or protected fingers to prevent Lyme disease, since *B. burgdorferi* takes several hours to a day or longer to pass from tick to host.

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