SHORT COMMUNICATION

Immunomodulatory arsenal of nymphal ticks

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Abstract. Ticks have developed their own immunomodulatory mechanisms to inhibit
the host inflammatory response. One of them involves the ability to subvert the cytokine
network at the site of tick feeding by secreting cytokine binding molecules. Most studies
have focused on the immunomodulatory prowess of adult female ticks. Here we de-
scribe anti-cytokine activity in salivary gland extracts (SGEs) prepared from 2-day-fed
nymphs of Dermacentor reticulatus Fabricius, Ixodes ricinus L., Rhipicephalus append-
diculatus Neumann and Amblyomma variegatum Fabricius. Anti-CXCL8 activity was
detected in nymphs of all species. Relatively high activity against CCL2, CCL3 and
CCL11 was observed in SGEs of R. appendiculatus and A. variegatum nymphs, whereas
SGEs of I. ricinus nymphs showed comparatively high anti-interleukin-2 (-IL-2) and
anti-IL-4 activities. These data show that nymphs, which epidemiologically are usually
more important than adults as disease vectors, possess a range of anti-cytokine activities
that may facilitate pathogen transmission.

Key words. Cytokine inhibitors, Ixodid ticks, nymph salivary glands.

Feeding ticks stay attached to their hosts for several days or
weeks, depending on the species and developmental stage. The
prolonged feeding period provides ample time for inflammation
to promote haemostasis at the feeding site. Host immune mech-


omics may reduce the feeding success of ticks by enhancing
inflammatory reactions. Ticks have developed mechanisms to
subvert the host response, presumably as an adaptation to obtain
larger bloodmeals that would result in increased tick fitness. In
particular, the saliva of ticks has anti-inflammatory and immu-
nosuppressive properties (Brossard & Wikel, 2004).

The host response to foreign antigens requires the co-ordinated
action of innate and acquired components of the immune system,
which is regulated by small secreted proteins known as cytokines
(Borish & Steinke, 2003). Cytokines are a diverse group of solu-
ble messenger proteins involved in the activation, growth, control
and repair of cells, and regulation of immune events. Chemok-
ines, a sub-set of cytokines, play an important role in controlling
leucocyte migration. In previous studies, saliva and/or salivary
gland extract (SGE) of ixodid (hard) adult tick species was shown
to bind numerous cytokines (interleukin-2, IL-4 and some impor-
tant chemokines) and suppress the activity of immune cells that
are responsive to their stimulation. Results varied between spe-
cies, and also between adult males and females of the same spe-
cies (Gillespie et al., 2001; Hajnická et al., 2001, 2005).

Manipulation of the host cytokine network by ticks provides a
mechanism to help ticks feed and may also facilitate tick-borne
pathogen transmission (Nuttall & Labuda, 2004).

The nymphal stage is often the most important in tick-borne
pathogen transmission. Several studies have shown that nym-
phal feeding induces changes to host haemostatic and immune
responses, with some evidence of differences between nymphs
and adults (Brossard & Wikel, 2004; Narasimhan et al., 2007;
Pedra et al., 2007). To determine whether nymphs have immu-
nomodulatory mechanisms similar to adults, we compared anti-
cytokine activity in SGEs prepared from nymphs of four ixi-
did tick species, Dermacentor reticulatus, Ixodes ricinus, Rhipi-
cephalus appendiculatus and Amblyomma variegatum, all of
which are important vectors of tick-borne pathogens (De Vos,
1981; Camus & Barre, 1992; Nambota et al., 1994; Hubalek
et al., 1997; Labuda & Nuttall, 2004; Foldvari et al., 2005;
Kelly, 2006; Sreter-Lancz et al., 2006; Skarphedinsson et al.,
2007). The materials and methods used followed those de-
scribed in our previous studies with adult ticks (Hajnická et al.,
2005; Vančová et al., 2006).

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Nymphs were allowed to feed on rabbits, on which complete engorgement takes approximately 5–8 days for D. reticulatus nymphs, 3–6 days for I. ricinus, 5–9 days for R. appendiculatus, and 5–9 days for A. variegatum (Honzáková, 1971; Jones et al., 1988). As anti-cytokine activity of ixodid species is most consistently detected after feeding commences but prior to engorgement, nymphs were collected when they had completed approximately 2 days of feeding (Vančová et al., 2006). Approximately 300 partially fed nymphs of each species were collected and SGEs prepared as described previously (Slovák et al., 2000). The total amounts of protein from 10 nymphs obtained from two independent feeding sessions were 2.8 µg and 3.9 µg in D. reticulatus, 5.1 µg and 3.5 µg in I. ricinus, 5.5 µg and 6.8 µg in R. appendiculatus, and 11.6 µg and 9.7 µg in A. variegatum. Pooled SGE was prepared as 10, 5, 2.5, 1 or 0.5 nymphal equivalents per 5 µl. Salivary gland extracts were screened by ELISA for activity against human CXCL8, CCL2, CCL3, CCL5, CCL11, IL-2 and IL-4 using commercial ELISA kits obtained from R&D Systems (Xxx) and/or Bender MedSystems Diagnostics (Xxx), as described previously, with duplicate assays of each sample (Hajníčkova et al., 2005). The results represent the means obtained with the two batches of SGEs derived from independent feeding sessions. A reduction in the detectable level of a particular cytokine, compared with the control, was interpreted as evidence of putative cytokine binding activity.

The SGEs of all the nymphal species reduced the level of CXCL8 (Fig. 1). The highest levels of inhibition were shown by R. appendiculatus and D. reticulatus. Thus there was no correlation between the total protein content of SGE from each species and the levels of inhibitory activity (D. reticulatus had the lowest protein content and R. appendiculatus the second highest). Relatively high activity against CCL2, CCL3 and CCL11 was observed in R. appendiculatus and A. variegatum nymphs, whereas activity was barely detectable in D. reticulatus and undetectable in I. ricinus. Only A. variegatum showed significant levels of activity with CCL5. Anti-IL-2 activity was detected in SGE of I. ricinus nymphs and low levels of activity in SGE of D. reticulatus nymphs, whereas anti-IL-4 activity was demonstrated in SGE of I. ricinus and R. appendiculatus. Thus nymphs of four ixodid tick species showed contrasting patterns of anti-cytokine activity after 2 days of feeding on rabbits. Similar results were obtained using murine (rather than human) cytokines (data not shown), reflecting the high degree of amino acid identity between mammalian cytokines and the likelihood that anti-cytokine activity is effective irrespective of (mammalian) host species.

For I. ricinus and A. variegatum, nymphal anti-cytokine profiles were similar to those recorded for adults, whereas adult D. reticulatus showed a much greater repertoire of anti-cytokine activity compared with conspecific nymphs (Table 1). The differences between species and between stages may reflect differences in host preference. However, I. ricinus has probably the most catholic ‘taste’, but appears to have the poorest anti-cytokine repertoire. An alternative explanation may be that anti-cytokine activity reflects the size of the mouthparts and/or the duration of feeding. For example, A. variegatum has large mouthparts that penetrate deep into the dermis, and takes a comparatively long time to reach engorgement (Stewart et al., 1998). The mechanics and physiology of A. variegatum feeding may antagonize different cytokines to those provoked by a species such as I. ricinus, which has much smaller mouthparts and engorges faster.

Soon after tissue damage, specific leucocyte subsets emigrate from the circulation into the affected area. These leucocytes function as the primary line of host defence in the destruction of micro-organisms and initiation of tissue repair. The histopathology of tick-bite lesions shows that, depending on the tick species and host species, the predominant cells infiltrating attachment sites are neutrophils (in mammals) or heterophils (in non-mammals), eosinophils and basophils (Latif et al., 1990; Szabo & Bechara, 1999; Van der Heijden et al., 2005). Neutrophils are the first infiltrating cell type in the dermis; their migration to inflammatory sites is directed by the chemokine CXCL8. The molecular structure of CXCL8 has been determined for various vertebrate species and shown to be similar. Indeed, the most ancient chemokine, found in a primitive group of vertebrates, resembles mammalian CXCL8, indicating high conservation of this chemokine since the evolution of early vertebrates (Najakshin et al., 1999). The chemotactic ability of human CXCL8 is not species-specific; granulocytes from many vertebrate species migrate to this chemokine in vitro (Rot, 1991). Polymorphonuclear neutrophils inform and shape immune responses. Thus it is perhaps not surprising that all the ixodid species and stages showed anti-CXCL8 activity, presumably indicating the importance to ixodid ticks of controlling neutrophil activity. Even adult female A. variegatum, which has low anti-CXCL8 activity at 5 days of feeding, shows comparatively higher activity earlier in feeding (Vančová et al., 2006).

The CC chemokines have pleiotropic activities; they are potent attractants for monocytes, eosinophils, basophils, natural killer cells and memory T cells (Laing & Secombes, 2004). The importance of cells of the host immune system infiltrating the tick feeding site resides in their ability to produce cytokines that modulate the downstream response (Falcone et al., 2001). Subversion of the activity of the four CC chemokines examined appears important for the two larger nymphal species, R. appendiculatus and A. variegatum.

Because of the relatively long duration of tick blood-feeding, ticks must suppress host immune reactions at all levels. The main function of IL-2 is to stimulate the growth and cytotoxic response of activated T lymphocytes. In addition, IL-2 is implicated in the development, homeostasis and function of natural killer cells. For nymphal I. ricinus in particular, the results suggest the importance of suppressing one or more of these functions.

The adaptive immune system has evolved two types of immune cells, Th1 and Th2, as the system supervisors (Kidd, 2003). Th1 cells are predominantly involved in the type-1 pathway of cellular immunity, whereas Th2 cells drive the type-2 pathway of humoral immunity. Th2 differentiation is a central process in the protection against parasites such as helminths. Tick infestation also results in a Th2 immune response, as shown by the cytokine profile induced in murine lymph node cells (Ferreira & Silva, 1999). IL-4 is a key cytokine in the induction of Th2 immunity, mediating B-cell activation. Activated
Fig. 1. Anti-cytokine activities of salivary gland extract (SGE) obtained from 2-day-fed nymphs of *Dermacentor reticulatus* (DR), *Ixodes ricinus* (IR), *Rhipicephalus appendiculatus* (RA) and *Amblyomma variegatum* (AV) ticks. Salivary gland extracts equivalent to 10.0, 5.0, 2.5, 1.0 or 0.5 nymphs (labelled 1, 2, 3, 4, 5, respectively) were pre-incubated with 50 pg of each cytokine for 90 min before ELISA analysis. Results are expressed as percentage reduction of OD reading compared with control.


Table 1. Host preferences of nymphal and adult tick species and their anti-cytokine activities.

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* Salivary gland extracts obtained from 2-day-fed nymphs. ++ +, 5%–25% ; ++ ++, 25%–50% ; ++ +++, 51%–75% ; ++++, 76%–100%. ND, not done.

Basophils with enhanced IL-4 production are candidates to mediate the innate-adaptive link for Th2 responses during helminth infections (Min et al., 2006). As the Th2 cells mature they also produce IL-4, which generates an autocrine loop to the naïve T cells to make more Th2 cells. As for IL-2, I. ricinus nymphs appear to target IL-4, suggesting they manipulate the Th2 response. When fed on laboratory mice, I. ricinus nymphs induce a Th2 response, but do not elicit a host rejection response (Brossard & Wikel, 2004). However, on natural hosts, I. ricinus nymphs induce resistance in Apodemus flavicollis, although not in Clethronomys glareolus (Dizij & Kurtnebach, 1995). Thus the interplay between tick-induced manipulation of host innate and acquired immunity, and Th1 and Th2 responses, for different host species, is highly complex. Identification of the tick molecules active in cytokine manipulation is needed to help unravel this complexity.

The importance of disrupting the host cytokine/chemokine network is demonstrated by the many ‘large’ DNA viruses that produce cytokine/chemokine inhibitors (Deane et al., 2000; Webb & Alcami, 2005). Arthropod-borne viruses (arboviruses) are ‘small’ RNA viruses (with one exception, African swine fever virus) and appear to exploit the immunomodulatory properties of their vector’s saliva (Nuttall & Labuda, 2004). Our results show that even nymphal ticks, which epidemiologically are generally more important than adults as vectors of arboviruses (and other tick-borne pathogens), produce a wealth of cytokine/chemokine inhibitors. Such important immunosuppressors will need to be considered in designing novel vaccines that target ticks and block arbovirus transmission.

Acknowledgements

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References


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