CMLS, Cell. Mol. Life Sci. 56 (1999) 286–295
1420-682X/99/040286-10 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Immunoglobulin-binding proteins in ticks: new target for vaccine development against a blood-feeding parasite

H. Wang* and P. A. Nuttall

NERC Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford OX1 3SR (UK), Fax + 44 1865 281696, e-mail: hwang@worf.molbiol.ox.ac.uk

Received 8 March 1999; received after revision 23 April 1999; accepted 26 April 1999

Abstract. Humans have a long history of trying to control ticks. At first, attempts focused on modifying the habitat, whereas later efforts relied heavily on the use of chemicals. Current research is directed at finding a vaccine against ticks. A strategy of targeting 'concealed antigens' succeeded with the first commercialised vaccine against the cattle tick *Boophilus microplus*. However, vaccine development against other tick species appears unsatisfactory to date. Vaccination depends on a specific antibody-mediated immunoreaction that damages the parasite. Immunoglobulin molecules of vertebrate hosts can pass through gut barriers into the haemolymph of ectoparasites while retaining antibody activity. Research on the ixodid tick *Rhipicephalus appendiculatus* revealed that host immunoglobulin-G in the parasite was excreted via salivation, during feeding. Immunoglobulin-binding proteins in tick haemolymph and salivary glands are thought to be responsible for such excretion. The discovery of an immunoglobulin excretion system in ticks indicates that they have a highly developed mechanism to protect themselves from their host's antibody attack. Such a mechanism questions whether immunization strategies will be effective against ticks, unless they circumvent or disable the ticks' immunoglobulin excretion system.

Key words. Immunoglobulin-binding protein; tick; salivary gland; haemolymph; gut; vaccine; tick-borne pathogen transmission.

Natural tick-host interactions

The suborder Ixodida comprises three tick families: the Ixodidae (hard ticks), the Argasidae (soft ticks) and the Nuttalliellidae. In total, there are approximately 820 tick species. Their huge geographical range and adaptation to climatic extremes (e.g. feeding on penguins in Antarctica and lizards in the tropics), and the diversity of hosts on which they feed (mammals, birds, and reptiles), show them to be a highly successful family.

Some tick species prefer to feed on particular vertebrate hosts, whereas other species feed on a range of hosts. Sonenshine [1] categorized the host selection of ticks as (i) host-specific (or host predilection) and (ii) opportunistic. The evolutionary history of the Ixodidae has been considered to relate to their host specificity. In long-established tick-host parasitic associations, some tick species developed certain mechanisms for suppressing the haemostatic and immune responses of their selected hosts [1]. Ribeiro [2, 3] remarks that ticks have a highly developed ability to evade and/or suppress host homeostatic systems, at least for the selected host species. Other tick species which lack the specific antagonists in their saliva that help evade the host immunological response may be rapidly rejected. In summary, ticks produce and inject saliva into the feed-

* Corresponding author.

ing site to alter their feeding environment, promoting continued blood flow by antihaemostatic and anti-inflammatory effects, thereby facilitating successful blood feeding. Constituents of saliva also minimise host immune responses, reducing the host's awareness of the parasite [4–8].

Opportunistic tick species are considered generalists, utilising a wide range of vertebrates as hosts. In nonspecific host-tick associations, many vertebrate hosts develop resistance (acquired resistance) to repeated tick challenge [9–12]. The naive hosts that have never been attacked by ticks are unable to resist the initial challenge, but they can establish an effective immune response to reject the ticks when challenged by the same species. In comparison with ticks feeding on naive hosts, fewer ticks feed on resistant animals, and they imbibe smaller bloodmeals. In addition, the moulting success and female reproductivity of the fed ticks is noticeably reduced [13]. Acquired immunity to certain tick species may vary among different host species.

The primary antigenic source that stimulates host immunity to ticks is saliva, the secreted product of tick salivary glands [14–17]. Salivary glands are the largest glands in the tick's body. As soon as feeding starts, ticks inject saliva into the host. Saliva from most ixodid ticks contains cement compounds that bind the tick's mouth parts to the host skin. Various enzymes and different types of bioactive molecules are also present in tick salivary glands and saliva [18–21]. These components are fundamental to obtaining a successful bloodmeal. They maintain blood flow into the wound (feeding site), antagonise host haemostatic and inflammatory mediators and help the tick to evade the host's rejection responses. Salivary gland constituents have been shown to modulate host cytokine responses and reduce lymphocyte responses to T cell mitogens [20, 22, 23]. Ticks can also secrete paralysing toxins via saliva that cause sickness or even death of their host [24]. The salivary gland is also the primary organ for tick-borne pathogen transmission [25]. Furthermore, it provides a saliva-activated transmission (SAT) activity [26], which plays an important role in nonviraemic tick-borne virus transmission [27, 28].

In summary, and as reviewed by Ribeiro [3], Wikel [13] and Nuttall [18], the tick-host molecular interactions are the result of tick-host coevolution. The host has evolved both innate and aquired immunity to counter tick infestation and feeding. In turn, the tick has developed adaptive mechanisms to protect itself and to minimise host immune reponses at the feeding site. A dynamic balance, between tick and host, achieves successful survival of any individual, either tick or host, in an established tick-host association.

Recent vaccine strategies against ticks

In 1939, Trager showed the anti-tick effect of injecting guinea pigs with extracts of whole larvae, salivary glands or digestive tracts of partially fed female ticks of *Dermacentor variabilis* (Say) [29, 30]. This early work included the two recent strategies that have been adopted for anti-tick vaccine development: (i) mimicking acquired resistance and (ii) targeting internal organs of the tick. Acquired host resistance against tick feeding is an immunological response to the tick saliva components. It is based on complement-dependent, cellular- and humoral-mediated effector mechanisms [13, 31]. Thus, salivary gland antigens were prepared and injected into hosts to stimulate acquired tick resistance. This strategy is not designed to damage tick salivary glands by specific antibodies. Instead, it aims to establish a rejective immunity that is triggered by the appearance of tick saliva in host skin when the tick feeds. Because a single, purified salivary gland antigen is unlikely to represent the immunogenic characteristics of natural saliva, mimicking natural acquired tick resistance appears to be difficult in practice and has been unsatisfactory to date.

Following the idea of a typical vaccine that induces a specific antibody to severely damage the parasite, antigens in tick internal organs (including salivary glands) were targeted. Kay and Kemp [32], and Willadsen [33] have reviewed these studies on arthropods, including ticks. To date, only the midgut Bm86 vaccine that affects *Boophilus microplus* (Canestrini) feeding on cattle has successfully passed all the tests and been commercialised in Australia [34] and Cuba [35]. Many vaccine projects against ticks have ended through lack of funding, although failure to consider the basics of tick physiology and biochemistry may also have led researchers to abandon ineffective strategies for tick control.

The fate of host immunoglobulin-G (IgG) in ticks

Immature and adult female ixodid ticks consume enormous bloodmeals, increasing their body weight more than 100-fold during feeding. The concentrated bloodmeal is slowly digested, primarily by an intracellular process (heterophagy) occurring within the midgut cells [19]. Not only does the tick retain potentially harmful immunoglobulins within the undigested bloodmeal, but a small but significant proportion of IgG and other host plasma proteins (e.g. albumin) cross the tick gut into the haemolymph [36–38]. Host IgG in tick haemolymph retains its biological activity [39], and specific antibodies can be detected that bind to internal organs (e.g. salivary gland and ovary) [40]. During feeding, the concentration of host IgG in ixodid tick

haemolymph increases [38]. Despite these observations, the fate of host immunoglobulins that entered the tick's haemocoel was previously unknown.

Studies on the uptake and persistence of ingested antibody in the mosquito, *Anopheles stephensi* showed that the level of antibodies in the haemolymph 24 h post-feeding was less than half the level in mosquito heads [41]. The authors postulated that antibodies were removed from the haemolymph by binding onto haemocoelic tissues. In ticks, four explanations can be considered to account for the fate of host immunoglobulins: (i) host immunoglobulins are absorbed by the tick haemocoel or body, (ii) the ticks gradually break down the harmful host proteins in the haemolymph, (iii) host immunoglobulins are removed from the haemolymph by the ticks or (iv) the ticks are inactive in dealing with host immunoglobulins.

Several apparently inexplicable observations have been recorded regarding IgG in ticks. In females of the argasid tick *Ornithodoros moubata*, the concentration of host IgG in haemolymph was reported to decrease from 10 ng/μl before feeding to almost zero after a bloodmeal, and then increased to greater than 10 ng/μl after 5 days following engorgement [42]. The authors suggested that the high IgG level in haemolymph before feeding originated from the last bloodmeal taken in the preceding nymphal stage. The fall in IgG titre during feeding, and the low level of IgG soon after feeding, was thought to result from a dilution effect as water from the bloodmeal was absorbed into the haemocoel. An alternative possibility, that the tick actively removed IgG from the haemolymph during feeding, was not considered.

In female *Amblyomma americanum* and *Dermacentor variabilis*, enzyme-linked immunosorbent assay (ELISA) data showed that the host IgG concentration in haemolymph generally increased during feeding [38]. However, in the early feeding period (weight range 4–60 mg), the IgG concentration in haemolymph actually decreased when ticks were fed on sheep or calves. The authors did not offer an explanation for this early drop in IgG levels. Again, it is possible that at least in the early stage of feeding, these ixodid ticks may have removed the IgG from their haemolymph.

Following the detection of host IgG molecules in saliva of the ixodid tick *Rhipicephalus appendiculatus* [43, 44], relatively higher concentrations of host (guinea pig) IgG were found in the saliva than in haemolymph and salivary gland extracts of partially fed female ticks [44]. When ticks were fed on guinea pigs immunised with killed *Escherichia coli*, 37% of the specific activity of the host serum IgG was retained by the IgG detected in the saliva. Similarly, 36% of the antibody activity was retained by IgG in salivary gland extract (SGE) and 42% in haemolymph [44]. Thus after passing through the gut wall, the host IgG molecules were apparently not subjected to further significant breakdown, but excreted by the feeding ticks via salivation. The salivary glands have been shown to exclude molecules the size of insulin (5 kDa) and polyethylene glycol (M_r 4000) [45]; hence it is unlikely that IgG in haemolymph diffuses out into the saliva in a nonspecific manner or passes out nonspecifically during maintenance of the water balance by tick salivary glands. If such nonspecific mechanisms occurred (or if IgG in saliva was due to contamination from the haemolymph during collection), other haemolymph proteins would also be present in sampled saliva, and consequently the relative concentration of IgG in saliva would be the same or less than in haemolymph when calculated by weight/weight of IgG/g of total protein. This was not the case, as the relative amount of IgG in saliva was >10 times that in haemolymph on day 4 and day 6 of feeding [44]. Furthermore, the protein profiles of saliva and haemolymph of the ticks were quite different [43]. Thus, these data were not consistent with leakage of immunoglobulins from the haemolymph through the salivary glands and into tick saliva, but suggested that the feeding tick excreted the host IgG via salivary glands. This mechanism could represent a self-defence system of the tick to protect it against the potentially harmful effects of immunoglobulins from immune hosts.

An additional reason for IgG excretion via saliva may be that the tick benefits by excreting IgG back into the feeding pool. Brown and Askenase [46] reported that immunoglobulin Fc receptors on host cells, such as mast cells and basophils, are required for antibody-mediated immune rejection of ticks from guinea pigs. Guinea pig recipients of anti-tick immune serum or immune peritoneal exudate cells expressed 25–30% tick rejection when challenged with *Amblyomma americanum* larval ticks. Pretreatment with either rabbit IgG or Fc fragments inhibited the expression of resistance by recipients of immune serum, but had no such inhibitory effect on the recipients of immune peritoneal exudate cells. Worms, Askenase and Brown [47] further reported that Fc receptors were required for guinea pigs to develop immune responses against *R. appendiculatus* ticks. If ticks excrete concentrated IgG, via saliva, back into the feeding site, the relative concentration of IgG in the tick biting site may be elevated. Such excreted IgG could compete for Fc receptors and consequently delay or reduce the normal immune response leading to rejection of ticks.

Immunoglobulin-binding proteins (IGBPs) in ixodid ticks

As discussed above, *R. appendiculatus* adult female ticks appear to excrete host IgG molecules back into their feeding sites via saliva. If this hypothesis stands, there must be some protein(s) in the tick that can recognise the IgG molecule and react to (i.e. bind to) it. When an IgG molecule passes though the tick midgut, it first enters the tick haemolymph and then finally is excreted by the salivary glands. Thus, in addition to salivary glands, IGBPs in ticks may also be expected in haemolymph. Moreover, IgG excretion in tick saliva might be expected to occur in several different tick species if it is a significant mechanism in ticks.

Using an IgG-linked agarose column system (fig. 1), we isolated numerous IGBPs from SGEs of the ixodid ticks *R. appendiculatus*, *A. variegatum*, *Ixodes hexagonus* [48] and *Ixodes ricinus* (fig. 2). Their molecular weights were identified by SDS-polyacrylamide gel electrophoresis (PAGE) (table 1). After cycling SGE in the column system, the first Sepharose column did not hold any salivary gland proteins, indicating that the proteins that appeared in the eluate of the second IgG-agarose column were affinity-bound to the IgG molecule and not due to nonspecific aggregation (fig. 2). Full-length recombinant proteins (IGBPMA and -MC produced in bacterial and baculovirus expression systems, respectively) were recognised by antisera prepared to the respective salivary gland-derived proteins, and vice versa, indicating that they were antigenically cross-reactive. However, when the recombinant proteins were applied to the column system, neither protein was retained. These observations indicate that the recombinant proteins, which were of low solubility, did not possess IgG binding activity. More important, the inability of the recombinant proteins to bind to IgG-agarose in the column system indicates that the binding observed with the salivary gland proteins was a specific reaction. Grubhoffer et al. [49] described lectins in the haemolymph of both ixodid (*Ixodes ricinus*) and argasid (*Ornithodoros tartakovskyi*, *O. papillipes* and *Argas polonicus*) ticks. The authors suggested that lectins in ticks play a role in self/non-self recognition. We did not find evidence that IGBPs are lectin-related. Elution of an SGE-treated guinea pig IgG-agarose column with 100 mM of either D-glucose, D-mannose, D-galactose, D-fucose or *N*-acetyl-D-galactosamine did not elute any of the IGBPs (unpublished data).

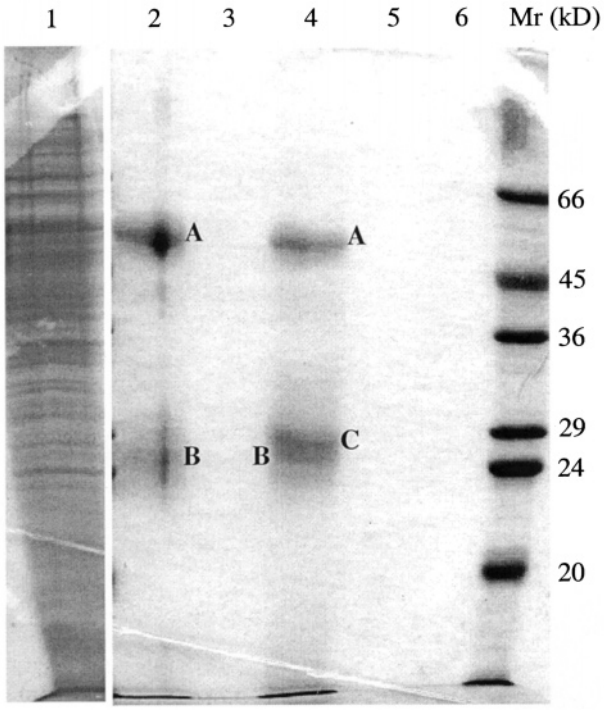

Figure 2. IGBP in salivary gland extracts of unfed *I. ricinus*. Lane 1, total protein profile of salivary gland extract; lane 2, protein background of the IgG-agarose column (see fig. 1, column 2). Bands A and B are the heavy and light chain of IgG, respectively; lanes 3 and 5, last washing fraction of the IgG and control columns, respectively; lanes 4 and 6, eluate (pH 2.6) of the IgG and control columns, respectively. The IGBP is marked as band C (27 kDa) in lane 4, but not found in lane 6.

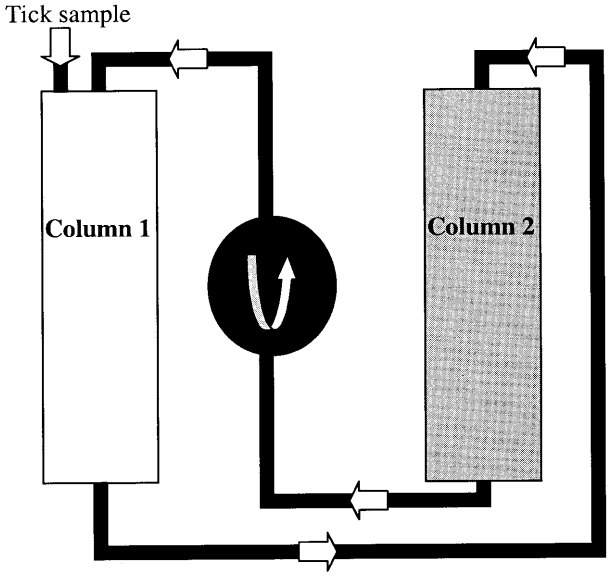

Figure 1. Affinity column system for isolating IGBPs. Column 1, control column of Sephadex-6B; column 2, affinity column of IgG-agarose. The two columns were eluted separately after washing steps.

In *R. appendiculatus*, two IGBP bands were detected in haemolymph collected from unfed females and males, and an additional 78-kDa IGBP was detected in haemolymph samples of male and female ticks that had fed for 6 days [48]. These haemolymph IGBPs may carry out the function of IgG recognition, whereas the salivary gland IGBPs may be responsible for IgG excretion. The 54-kDa IGBP from female [50] and 21-kDa from male [51] SGEs of day 6-fed *R. appendiculatus* ticks were able to bind IgG of guinea pigs, on which host the tick colony was maintained, and also other mammalian IgGs tested (human and bovine). Although IGBPs vary in different tick species (at least in size), it appears that the general mechanism of clearing host IgG is widespread among ixodid ticks. The existence of IGBPs in unfed ticks also suggests that this mechanism may function as soon as blood feeding commences.

Male IGBPs in *R. appendiculatus*

In the adult stage, male metastriate ticks (but not prostriate ticks) require a bloodmeal for their maturation. Female ticks imbibe up to 100 times more blood than the males, during the 1- to 2-week feeding period. After engorgement, female ticks lay a large egg mass (up to 20,000 eggs) and then die. Male ticks take relatively much smaller amounts of blood (increasing their body weight some 1.5 times) during feeding. After mating with the females, the male ticks feed together with their mates. When *R. appendiculatus* is fed on guinea pigs, the male and female ticks first feed separately for a few days, then the male detaches and moves to the feeding female tick, mates and cofeeds adjacent to the female. When the female ticks have engorged and dropped off their host, the males may remain on the host waiting and searching for other females.

Male *R. appendiculatus* ticks have a 54-kDa salivary gland IGBP similar to that of the females. However, unlike the female IGBP that binds IgGs from guinea pig, human and bovine hosts, the male 54-kDa IGBP does not bind to bovine IgG [51]. This variation in IgG-binding host specificity suggests that the IgG-excreting mechanism may differ between conspecific male and female ticks. Indeed, the 54-kDa IGBP is not the most abundant IGBP in the male salivary glands, whereas it is the major IGBP band in the female SGE. Three more abundant IGBPs are present in the partially fed male SGE [51]. The 21-kDa band, designated IGBPMC, bound to IgG of three potential host species (guinea pig, human and bovine), whereas the 29-kDa IGBPMA did not bind to bovine IgG. It was not clear whether the 25-kDa IGBPMB could bind to human or bovine IgG, because it migrated in the gels in the same position as the light chain of the respective IgG. All three IGBPs (-MA, -MB and -MC) were specific for partially fed male ticks; they did not cross-react antigenically with any female SGE proteins. The appearance of abundant IGBPs in the feeding male salivary glands strongly suggests that the male ticks have developed a male-specific mechanism to protect themselves against the potentially harmful host immunoglobulins.

Following the cloning of full-length encoding sequences of the abundant male IGBPs (-MA, -MB and -MC) from a λ-complementary DNA (cDNA) library of partially fed male tick salivary glands, the amino acid sequences were determined (GenBank accession numbers AF001868, AF001869 and AF001870 for IGBPMA, -MB and -MC, respectively). Sequence analysis showed that IGBPMB and -MC are related proteins (50% identity and 70% similarity) with six conserved cysteines (fig. 3), and have distant homology to a mammalian transport protein, ganglioside GM2 activator. IGBPMA is not related to -MB or -MC. The sequence homology of IGBPMB and -MC indicated that these two proteins are involved in the same function for male ticks, i.e. binding to host IgG during feeding. Both IGBPMB and -MC have potential asparagine (N) glycosylation sites. Treatment with N-glycosidase F (PNGase F) revealed that an N-linked

Table 1. Molecular weights (kDa) of IGBPs in SGEs of ixodid ticks

Ticks					
R. appendiculatus			*A. variegatum*	*I. hexagonus*	*I. ricinus*
Unfed	fed		unfed	unfed	unfed
	female	male			
54	54	54	47	41	27
45	45	29	16.5	15	
36	36	25	15.5		
22	22	21	14.5		
21	21				

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Percent Similarity: 70.238   Percent Identity: 50.595

Igbpmb.Pep x Igbpmc.Pep
                .         .         .         .         .
IGBPMB   1 MTFAIVAVLLGSLGGGPETPVQYHPMLRCDTDHAAEIMRVTTKEAVVNST 50
           |.||: ||||:  ...:..:||||.:::|. :..|||| |.|::|||.
IGBPMC   1 MMSFAVSAVLLAFAVNVAHGAVQYHQLIKCSQNYSAEIMGVVTRDAVVGHQ 51
                .         .         .         .         .
          51 MKAKINVRVYDAILKDLKLRLALFTPEGHIVPCINGFGSCVYDLCK..PN 98
             |.|||.:|||||.|||:||:|:||||||:| :||||:|||||||||:|.  |:
          52 MTAKIKLRVYDTILKELKMRVALFTPQGTVVPCIEGFGSCVYDVCEDVPE 101
                .         .         .         .         .
          99 KNTTMLLGNGTQANNGAQCPVPPGTYYRTLDFKLSPELNKHIGNGELLGT 148
             ...||:          ...|||.||||:|.| |::|| : |||||||:|::.
         102 NKVTMW.........TTKCPVKPGTYWRNLVFRVSPRMLKHIGNGNLIAA 142
                .         .
         149 MQLESGNKVVACHAYFMVLYSKRPKAKEW 177
             : ||| .| :.|:|. : ::..||... |
         143 LMLESKGKKLSCQALHLRVFKQRPTTDIW 171
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Figure 3. Bestfit analysis of the full-length translated amino acid sequences of cDNA clones of IGBPMB and -MC. Underscore indicates the signal sequence of IGBPMC.

oligosaccharide chain is bound to the asparagine residue at position 33 of the IGBPMC. Deletion of this oligosaccharide chain reduced the molecular weight of the recombinant IGBPMC by 3.5 kDa, as determined by SDS-PAGE. PNGase F digestion also caused a reduction in the molecular weight of tick-derived IGBPMC to the same size as the recombinant IGBPMC, and also shifted the tick-derived IGBPMB to a smaller size, indicating that both native IGBPMB and -MC are glycosylated. Tick-derived IGBPMA did not shift to a smaller size in SDS-PAGE after PNGase F digestion.

Even though IGBPMB and -MC are related proteins, they are antigenically distinct as determined for tick-derived proteins using immunoblotting [51], and confirmed by tick-feeding experiments [52]. The 'antigenicity' analysis in the GCG (Genetics Computer Group) package predicted that the only antigenic regions that may be similar between the two are PGTYY (amino acids 121–125) of IGBPMB, and PGTYW (amino acids 115–119) of IGBPMC. Interestingly, in the most conserved region (fig. 3, amino acids 51–96 of IGBPMB and 52–97 of -MC, 71.7% identity and 89.1% similarity), IGBPMC does not have any predicted antigenic sequence. Both sequence analysis and experimental data indicated that IGBPMC is a secreted protein, whereas IGBPMB lacks a signal sequence, implying that IGBPMB is not secreted in tick saliva. Thus, the distinct antigenicities of IGBPMB and -MC may have biological significance. Ticks may only expose the secreted proteins to the host and hide the antigenic properties of nonsecreted proteins so that the host-derived defensive mechanism will not be able to damage the unsecreted proteins and thereby disturb tick salivary gland functions, e.g. excreting host immunoglobulins by IGBPs during feeding.

Recombinant IGBPs were used to immunise guinea pigs, and then ticks were tested on these immunised hosts for tick-feeding performance. Guinea pig antisera raised against the recombinant IGBPMB (from pGEX/*E. coli* expression) and -MC (from baculovirus expression) developed single bands of tick salivary gland-derived -MB and -MC, respectively. When adult *R. appendiculatus* ticks were fed on the IGBPMC-immunised guinea pigs, none of the ticks died. Similarly, the survival time of male ticks post-engorgement was not effected by immunisation. However, the IGBPMC vaccine resulted in a delay in the time taken by female ticks to complete engorgement. The IGBPMB vaccine did not have the same effect on adult female ticks (fig. 4). Considering that the male adult ticks take a relatively small bloodmeal and that, consequently, sufficient specific antibody may not reach the target proteins in salivary glands to exert an effect, anti-IGBPMC serum was injected into male ticks at day 4 of feeding in situ. Male ticks survived injection with the antiserum. Female ticks showed a significant reduction in engorged body weight and egg mass when they fed together with the anti-IGBPMC serum-injected males. The tolerance to the anti-IGBPMC treatments by the male ticks indicated that IGBPMC is not essential for male tick survival. More important, the apparent effect of the antimale IGBP treatments on female ticks indicates that male ticks not only feed with female ticks for copulation

but that male ticks also help female ticks to complete successful engorgement [52]. Thus, in addition to the function of excreting immunoglobulins, the secretory male IGBPMC helps the female ticks to feed, presumably by impairing immunoglobulin-mediated host reactions against tick feeding. If secreted IGBPs are able to block the interaction between tick-specific IgG and effector cells, at the site of feeding, they may suppress antibody-dependent cellular cytotoxicity. Other parasites have targeted their hosts' immunoglobulin system, focusing on the Fc fragment of IgG [53–57]. The means by which ticks interact with IgG has yet to be determined.

New strategy for anti-tick vaccine development

As described above, ticks have developed a mechanism for excreting host IgG via their salivary glands. This mechanism may minimise the potential damage caused by host antibody during feeding, particularly in adult female ticks. Male *R. appendiculatus* secrete IGBPMC into the female feeding site to help their mates feed. The IGBP-mediated 'mate guarding' suggests that the male tick not only protects itself from host immune rejection during feeding but has also evolved secretory IGBPs that actively target antibody-mediated immune responses to modify the feeding site, so that its mate can successfully complete engorgement and produce offspring. Thus, even when intact host IgG molecules pass through the tick gut barrier into the haemolymph, the ability of antibody to damage internal tick organs is questionable. The IGBPs in tick haemolymph may react with any host antibody that passes through the gut wall and transport the antibody to the salivary glands. IGBPs in salivary glands then excrete the antibody back into the feeding site. By means of the tick immunoglobulin excretion system (TIES), host immunoglobulin is actually controlled during its passage through the tick's body and may not damage the tick (fig. 5). Therefore, in order to control ticks by host antibody derived against tick internal organs, the TIES obviously needs to be disabled or avoided.

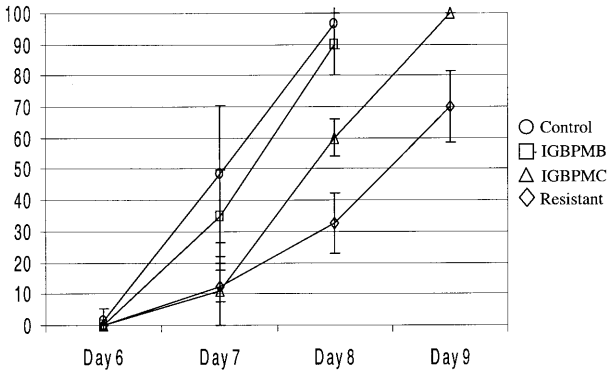

Figure 4. Comparison of engorgement times of female *R. appendiculatus* feeding on guinea pigs. Control, naive guinea pigs (60 female ticks, 6 guinea pigs); IGBPMB, recombinant IGBPMB-immunised guinea pigs (20 female ticks, 2 guinea pigs); IGBPMC, recombinant IGBPMC-immunised guinea pigs (20 female ticks, 2 guinea pigs); resistant, tick-resistant guinea pigs (30 female ticks, 4 guinea pigs).

The first commercialised anti-tick vaccine targets the tick gut, thus avoiding the TIES. Ticks fed on Bm86-vaccinated hosts died from severe antibody-mediated damage to the gut. Surviving ticks also suffered from a reduction in engorgement weights and egg-laying capacity [34, 58]. The protective antigen, Bm86, was originally identified in partially fed adult *Boophilus microplus* as a membrane protein of gut digest cells. The tick *B. microplus* is a one-host tick, i.e. the engorged larvae and nymphs do not drop off the host. Larvae take 3 weeks to become the engorged adult. Although the vaccine efficacy for controlling immature *B. microplus* is uncertain, the Bm86 vaccine clearly demonstrated that targeting the tick gut alone can cause severe damage to the tick. The vaccine can be further improved when used in combination with other concealed antigens, such as Bm91 and BMA7 [59, 60]. Bm91 is a carboxydipeptidase, largely concentrated in the salivary glands [61]. Antigen BMA7 is a mucin-like membrane glycoprotein widely distributed in the tick body [60]. Vaccination using Bm91 or BMA7 alone was not as effective as the Bm86 vaccine, but combinations with Bm86 significantly enhanced vaccine efficacy. These two recent combined Bm86 vaccine trials signalled an improved two-step strategy for tick control involving (i) damage to the tick gut by an antibody to allow (ii) a second antibody to pass efficiently through the gut and into the haemolymph to target internal organs. Sauer, McSwain and Essenberg [62] reviewed key internal proteins that can be targeted for developing an anti-tick vaccine. The idea of using combined antigut vaccine antigens makes targeting internal key proteins a realistic possibility.

An alternative strategy is to kill ticks after feeding rather than trying to kill ticks during feeding. Adult feeding causes most of the tick damage to the host. If ticks can be killed at the immature stages, there will be a significant increase in the economic effectiveness of anti-tick vaccines. Nymphal tick blood feeding is similar to the mechanism of engorgement by adult females. Thus, a successful antinymph gut vaccine may severely damage nymphal gut when ticks engorge on the vaccinated host. A combined second vaccine which targets a key component of the moulting tick may kill the engorged nymphs that survived from the antigut vaccine, during the moulting stage, thus protecting the host from the much more damaging effect of adults.

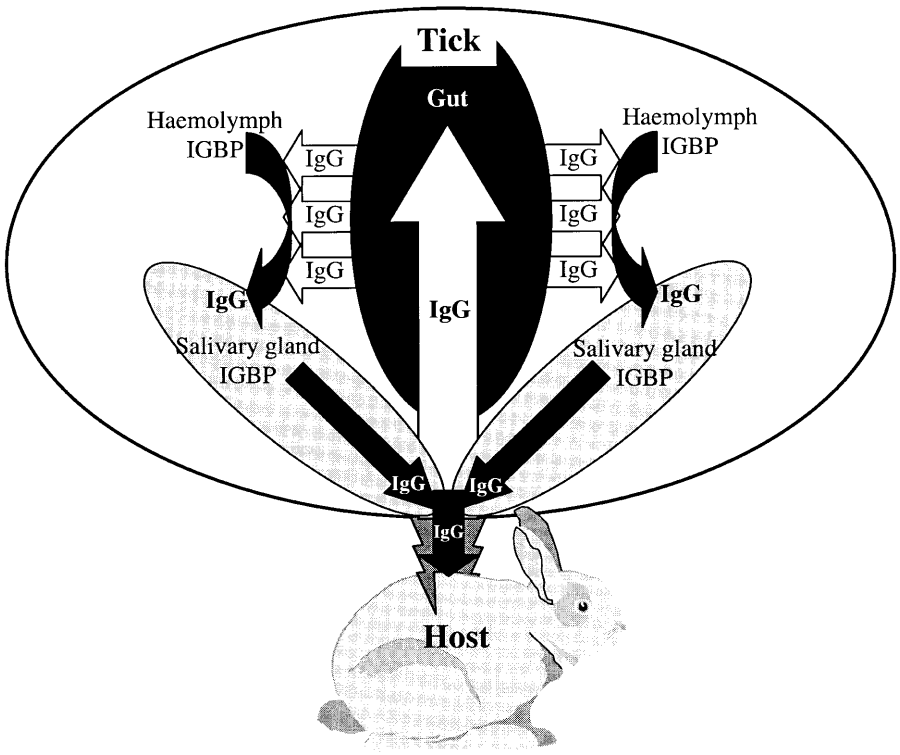

Figure 5. Model of the proposed fate of host IgG in ticks. Intact host IgG is uptaken into the gut by a feeding tick, and passes through into the haemolymph (clear arrows). Then the IgG molecules are bound, transported and finally excreted back in to the tick feeding site by IGBPs in haemolymph and salivary glands (black arrows).

The tick self-defence system, particularly the TIES, needs to be considered for achieving maximum vaccine efficacy. The functional immunoglobulin excreting system pumps host antibody out of the tick body during feeding. Stopping the function of the TIES will increase the host antibody concentration in the tick body, thus damaging the tick more efficiently, and may consequently kill or induce rejection of the tick in the early feeding stage. The IGBPs in tick haemolymph and salivary glands may be targeted directly. However, it is likely that there is generally more than one IGBP species in an individual tick, and they are not minor proteins. More detailed studies are needed to determine the mechanism by which IGBPs recognise, transport and excrete immunoglobulin. In *R. appendiculatus*, the IGBPs in haemolymph seem better targets than those in the salivary glands because (i) the IGBP species are shared by both male and female ticks, (ii) they are less abundant in haemolymph than in the salivary glands and (iii) they are more likely to be 'concealed' antigens. More important, when bound by the haemolymph IGBP, the host antibody may become inactive even though it remains in the tick body. Thus, the haemolymph IGBP may in general also be a more effective target than the salivary gland IGBP for a vaccine.

Using concealed antigens to protect hosts against ticks is a successful strategy for vaccine development. Theoretically, concealed antigens that are never exposed to the host imply that ticks are less likely to have evolved ways of evading the host immunological response to these antigens. In accord with what little we know about the TIES, and the effectiveness of combined antigut tick vaccines, the most effective anti-tick vaccine should open (damage) the gut first, stop the TIES functioning and target a key component to kill the tick quickly. Modern molecular biology approaches offer a variety of techniques to produce a recombinant immunogen that displays antigenic conformation and epitopes from different origins. Will the combined vaccine strategy win us the long battle against ticks? Or will this very successfully evolved ectoparasite hit back with another unexpected adaptation. Only time will tell. Greater understanding of the general biology of parasites, particularly their self-defence systems and strategies, is needed if we are to compete with the evolutionary power of parasites.

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