RECENT VACCINE RELATED STUDIES WITH ECONOMICALLY IMPORTANT GASTROINTESTINAL NEMATODE PARASITES OF RUMINANTS

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Abstract. Although several native antigens of sufficient efficacy to be commercially useful have now been discovered for *Haemonchus* and *Ostertagia*, all will have to be synthesised artificially to be economically viable. Despite numerous attempts, recombinant DNA technology has not yet yielded the solution, but the effort continues, spurred on by the successes with cestodes and certain ticks. *Teladorsagia* and *Trichostrongylus* lag behind in the vaccine research stakes: here, the first and probably most difficult step of identifying a reliably protective native antigen extract does not seem to have been achieved yet. It may be necessary to stimulate elements of the mucosal response to induce protection, a subject still in its infancy as far as ruminants are concerned. Several laboratories have started to work on antigen delivery methods with this in mind. It is easy to understand why intestinal antigens protect against blood feeding *Haemonchus* rather than mucosal browsers, but quite why conventional immunisation works for *Ostertagia,* but not apparently for *Teladorsagia* or *Trichostrongylus* remains a mystery.

INTRDOUCTION

Since the fourth Novel Approaches conference in January 2005, the subject of immunity and or vaccination in relation to gut nematodes of ruminants has been reviewed at least 5 times ([Hein & Harrison, 2005; Bethony *et al*., 2006; Miller & Horohov, 2006; Smith & Zarlenga, 2006; Vercruysse *et al.,* 2007). As the title suggests the present brief review concentrates on efforts during that period to vaccinate ruminants against gastrointestinal nematodes. No attempt will be made to be comprehensive, rather there will be inevitable bias towards those parts of the topic the author knows best!

Readers looking for similar information on flukes, cestodes or lungworms in the same hosts are directed to reviews by McManus & Dalton (2006); Lightowlers (2006a, 2006b) and McKeand (2000) respectively.

The state of play with four important species

Haemonchus contortus

Despite the fact that protective excretory / secretory and larval somatic antigens have been described before e.g.(Vervelde *et al*., 2001) a literature search revealed that all *Haemonchus* vaccine publications since 2005 were concerned with antigens derived from adult worm intestinal cells. Of these, H11 and H-gal-GP have been the most extensively characterised and to date remain the most protective defined antigens identified for any nematode species of any host (Smith & Zarlenga, 2006). For example, a field trial conducted with Merino weaners in a *Haemonchus* endemic area of Australia showed that a combination of these antigens could be extremely effective. Deaths, anaemia,

faecal egg counts and subsequent pasture contamination were all substantially reduced in the vaccinated sheep compared to those which received adjuvant alone. It was concluded that if the same effects could be reproduced with recombinant versions of these antigens, the prospects for a *Haemonchus* vaccine were good. However, results from a separate trial with housed sheep did not support the possibility that this type of vaccine could also act synergistically with anthelmintic drugs (Smith, 2007).

H11 is a family of aminopeptidases present on the intestinal brush border of *Haemonchus*. Despite extensive, largely unpublished efforts in Australia in recent years, little or no protection has been achieved with recombinant versions of these antigens, even when administered as a cocktail of four enzymically active members of this family (Sexton & Zawadzki personal communication). Potential explanations for this surprising failure have been debated before (Newton & Meeusen, 2003; Smith & Zarlenga, 2006). It was surprising therefore when partial protection against worm numbers (egg counts were not presented) was recently claimed in a single experiment in which five sheep were immunised with a recombinant version of one member of this aminopeptidase family (Reszka *et al*., 2007). It is important to determine whether this result can be confirmed.

H-gal-GP is another intestinal cell brush border complex, although here the protective components consist of a family of four metalloproteases combined with a family of two aspartyl proteases (Smith *et al.,* 2003a; Smith *et al.,* 2003b; Newlands *et al*., 2006). Like H11, the native complex loses most of its protective activity if it is dissociated, which indicates that conformational epitopes are important and explains why bacterially expressed insoluble recombinants do not work (Smith *et al.,* 2003a; Smith *et al.,* 2003b). Most of the H-gal-GP enzymes have now been expressed in eukaryotic systems as soluble proteins and a cocktail of these is currently being evaluated in a sheep protection trial.

The 3-D structure of H-gal-GP complex has recently been visualised by electron microscopy (Meunch *et al*, poster presented at US Biophysical Society, San Diego, February 2008). The complex's shape is unique and intriguing particularly as it contains an internal chamber with three openings. Since vaguely analogous complexes which function as protease "machines" have been described in bacteria (Tamura *et al*., 1996; Franzetti *et al.,* 2002), it is hypothesised that the structure of the complex has evolved to efficiently dock and then digest protein substrates in the parasite's blood meal. Although more work is needed to elucidate the stoichiometry of the complex, its quaternary structure goes a long way to explain why unfolded recombinant versions of its sub-units do not protect and suggests that alternative approaches to producing a synthetic H-gal-GP vaccine may be needed.

Vaccination with native membrane-bound cysteine proteases from adult *Haemonchus* has been shown to confer significant levels of protection against homologous challenge in sheep (Knox *et al*., 2005). Sheep vaccinated with solubilised and re-folded recombinant versions of these enzymes showed modest (29 - 38%) but significant reduction in worm burdens, though, curiously, faecal egg counts were unaffected (Redmond & Knox 2004; Redmond & Knox 2006). Methods have recently been devised for expressing a related *Haemonchus* cysteine protease as an active recombinant enzyme secreted by *Caenorhabditis elegans* (Murray *et al*., 2007). Hopefully this technology can be used generically to produce membrane-bound cysteine proteases and other candidate protective antigens, all folded and glycosylated in a manner closely resembling the parent form.

Galectins are beta-galactoside-binding proteins detected in several species of ruminant gastrointestinal nematodes (Greenhalgh *et al*., 2000). Members of this family have been isolated from the intestinal cells of adult *Haemonchus* (Newlands *et al.,* 2006). Recently it was claimed that goats immunised with two *Haemonchus* recombinant galectins were partially protected against challenge (Yanming *et al*., 2007), a result which contradicted an earlier trial with sheep where the immunogen was a mixture of several native

galectins(Newlands *et al*., 2006). This is another discrepancy in the literature which needs to be resolved.

The question as to whether *H.contortus* gut membrane glycoproteins like H11 and H-gal-GP are "hidden", ie. not recognised by the host during infection, has been the subject of some debate (Smith, 2007). The "hidden" versus "natural" antigen concept is not just academic because, if natural boosting occurred, the duration of vaccine immunity with "hidden" antigens could be greatly increased. *Haemonchus* gut membrane proteins were originally described as "hidden" because immune sheep which had been exposed to prolonged infections did not recognize them serologically (Smith, 1993), but contradicting data has since been published (Jasmer *et al*., 1993; 2007). However, in field trials where vaccinated sheep were continuously exposed to infection, incoming larvae did not maintain the high concentrations of circulating antibody needed to maintain protective immunity (Jasmer *et al*., 1993; Smith *et al*., 2001b). Similarly, in a trial designed to measure the duration of immunity in sheep immunised with H-gal-GP, there was no hint of a post challenge secondary antibody response in vaccinated sheep (Sherlock, P. MSc thesis University of Edinburgh, 2006). Therefore from a practical viewpoint these antigens behaved as if they were "hidden". Clearly, more work is required to understand the mechanisms behind this apparent contradiction.

b) *Teladorsagia circumcincta*

To the author's knowledge no consistently effective protective antigens have ever been described for this parasite, despite several trials with different candidates (Morton *et al*., 1995; Smith *et al*., 2001a). For example, recent attempts to immunise sheep with either native excretory-secretory (ES) products from fourth stage larvae or with recombinant cathepsin F (Redmond *et al*., 2006) a naturally immunogenic ES component (Halliday *et al*., 2007), did not result in any protection despite high titre serum antibodies being stimulated (Matthews et al personal communication).

c) *Trichostrongylus colubriformis*

As with *Teladorsagia*, no protection trials with *Trichostrongylus colubriformis* candidate protective antigens seem to have been published since 2005. This is despite the discovery of CarLa, a promising carbohydrate surface antigen, which seems to be the target of a mucus IgA response that prevents larval establishment (Harrison *et al*., 2003a; 2003b). Two further predominantly carbohydrate antigens, present also on *Haemonchus* and *Teladorsagia* L3s, have been described since (Maass *et al*., 2007), but again no data on their protective capacity seems to have been published. It would be surprising if a simple conventional vaccine trial has not been attempted with any of these. Hopefully some of our New Zealand colleagues at NA5 will enlighten us on the outcome.

d) *Ostertagia ostertagi*

Unlike the other two non blood feeding species just listed above, considerable activity with potentially protective *Ostertagia ostertagi* antigens has been published in the last three years.

Attempts to immunise calves with a globin-enriched fraction from adult worms gave highly variable levels of protection (Claerebout *et al.,* 2005). Similarly, neither an aspartyl protease inhibitor, which is recognised serologically by naturally immune cattle (De Maare *et al.,* 2005), nor a recombinant *O. ostertagi* heat shock protein (Vercauteren *et al*., 2006), offered any vaccine promise. However, a thiol binding fraction prepared from the ES of adult parasites was shown to reduce *O. ostertagi* faecal egg counts by some 60%, though the choice of adjuvant proved critical (Geldhof *et al*., 2004). Subsequent fractionation of this preparation, which is enriched for activation associated secreted proteins (ASPs) and cysteine proteases, revealed that both these components were equally protective, giving a 70-80% reduction in cumulative faecal egg count. Interestingly, a third sub-fraction containing the rest of the preparation was just as effective (Meyvis *et al*., 2007).

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