

Project number: 5685
Funding source: Teagasc

Date: September 2015
Project dates: Mar 2007-Nov 2011

The association of loci of the MHC complex with genetic resistance/susceptibility to gastrointestinal nematodes in sheep



Key external stakeholders:

Sheep producers, helminthologists, immunologists, geneticists, veterinarians, agricultural advisors

Practical implications for stakeholders:

This study has confirmed the involvement of the *Ovar-DRB1*0203* allele in resistance to *T. circumcincta* in Suffolk sheep.

- The *Ovar-DRB1*0203* allele had a significant effect on response to nematode infection with the carriers of this allele having lower worm numbers than the non-carriers.
- The efficient immune response, particularly the switch from a Th1 to the anti-parasitic Th2 response; both at the cellular and humoral level appear to be a key mechanism underlying this resistance.
- Additionally, this study shows that the upholding of gastrointestinal integrity, reduced stress-related cell turnover and nutrient utilisation are some of the processes driving resistance associated with the *Ovar-DRB1*0203* allele.

Main results:

- This study has confirmed the involvement of the *Ovar-DRB1*0203* allele in resistance to *T. circumcincta* in sheep. The *Ovar-DRB1*0203* allele had a significant effect on response to nematode infection with the carriers of this allele having lower worm numbers than the non-carriers. However, No difference was observed in FEC
- The cytokine gene expression studies that the *Ovar-DRB1*0203* carriers exhibited an early switch from a T-helper type 1, characterised by expression of IL-1 β , and IFN γ , to a T-helper type 2, exemplified by the expression of IL-10, and IL-13, immune phenotype. The efficient immune response, particularly the switch from a Th1 to the anti-parasitic Th2 response; both at the cellular and humoral level appear to be a key mechanism underlying this resistance.
- Higher mast cell counts were observed in the abomasal mucosa, while higher platelet counts were present in the carriers compared to the non-carriers. Similarly, enzyme-linked immunosorbent assay indicated higher levels of mucosal IgA and IgE in the carriers than the non-carriers.
- Using 2D-gel electrophoresis, differential protein expression was observed in the mucosa of these lambs with the carrier lambs showing an increased expression of immune related proteins e.g. lysozymes, while the non-carrier exhibited increased expression of energy utilisation proteins e.g. triosephosphate isomerase-1. The expression of these proteins has
- Results of the study indicated that the upholding of gastrointestinal integrity, reduced stress-related cell turnover and nutrient utilisation are some of the processes driving resistance due to the *Ovar-DRB1*0203* allele.

Opportunity / Benefit:

This research has advanced our knowledge on the role of the *Ovar-DRB1*0203* allele on resistance to gastrointestinal nematodes in sheep and the biological mechanisms underlying the resistance. It has confirmed the possibility of breeding sheep for increased nematode resistance and established a basis for future research on the mechanisms underlying gastrointestinal nematode resistance.

Collaborating Institutions: UCD

Teagasc project team: Dr. Barbara Good (PI)
Musa Abdul Hussan (WF)
Dr. JP. Hanrahan
Henry Walsh
Assumpta Glynn

External collaborators: Prof. Torres Sweeney (UCD)
Prof. Grace Mulcahy (UCD)
Dr. Deirdre Campion (UCD)
Dr. Gearoid Sayers (UCD)

1. Project background:

The abomasal worm *Teladorsagia circumcincta* (*T. circumcincta*) is amongst the most important parasites affecting livestock production and predominates in cool temperate areas. While control of these parasites has principally been achieved by the use of anthelmintics, the emergence of parasites resistant to anthelmintics has made reliance on these drugs unsustainable. Genetic selection for parasite resistance in domestic sheep is being promoted to minimise the use of chemoprophylaxis. To this end, several genetic markers of resistance have been identified, including an allele (*Ovar-DRB1*0203*) of the major histocompatibility complex DRB1 (MHC-DRB1). For instance, Suffolk sheep carrying the ovine-*DRB1*0203* allele have been reported to show increased resistance to natural *Teladorsagia circumcincta* infection compared to non-carriers. However, the biological mechanisms by which these markers exert their influence are not known. In the present study, a group *Ovar-DRB1*0203* carrier and non-carrier twin-pair lambs, was used to investigate the relationship between this locus and the physiological and immunological processes driving resistance to *T. circumcincta* such as, smooth muscle contractility, abomasal mast cell hyperplasia and production of IgA and IgE.

2. Questions addressed by the project:

- What are the biochemical and physiological responses of *Ovar-DRB1*0203* carrier and non-carrier twin lambs to an experimental infection with of *Teladorsagia circumcincta*?
- What are the cytokine gene expression response profiles in the abomasal mucosa of these lambs during the course of infection and is this affected by genotype?
- Would proteomic analysis of the abomasal mucosa reveal protein expression profiles underlying the differential response to *T. circumcincta* between *Ovar-DRB1*0203* carrier and non-carrier lambs and identify potential effector molecules driving local response to gastrointestinal nematodes?

3. The experimental studies:

Experiment 1

A total of six sires heterozygous for the *Ovar-DRB1*0203* allele were mated with ewes in single sire groups to generate progeny carrying or lacking the *Ovar-DRB1*0203* allele. All lambs were genotyped at the *Ovar-DRB1* locus and twin pairs consisting of a carrier and non-carrier identified for use in this study. The lambs were born indoors at the Teagasc Animal Production Research Centre, and were put to pasture until they were between 4 and 5 weeks of age when they were moved back indoors with their dams. All lambs were weaned 1 week later and faecal sampled and treated with Oramec (Merial Animal Health Ltd) as per the manufacturer's instructions. All lambs were free of nematode infection at the start of the experiment, based on FEC measurements on 3 consecutive days. At about 12 weeks of age, eight lambs (4 twin pairs; each pair consisting of an *Ovar-DRB1*0203* carrier and non-carrier) were slaughtered (day 0; controls) and the remaining lambs each received a single oral dose of 30000 infective *T. circumcincta* L3. On days 3, 7, 21, and 35 post infection eight lambs (4 twin pairs; each pair consisting of an *Ovar-DRB1*0203* carrier and non-carrier) were slaughtered. The choice of these time-points was based on the developmental stages of the parasite in the sheep abomasum, Day 3 (L3 stage), Day 7 (L4 stage) and Day 21 and 35 (egg laying stage). The variables studied included worm burden, faecal egg count, abomasal mast cells, IgA, IgE, IgG1/IgG2 and haematological parameters at 0, 3, 7, 21 and 35 days post infection, and duodenal smooth muscle contractility at 0 and 35 days post infection.

Experiment 2

The twin pair lambs carrying or lacking the *Ovar-DRB1*0203* allele were used in a time-course cytokine gene expression assay to determine if resistance to gastrointestinal nematodes is due to an early switch from Th1 to Th2 mucosal immune response. Mucosal scrapings from the abomasal tissue removed from challenged

and unchallenged lambs were preserved in RNeasy[®] (Applied Biosystems, Warrington, UK) and stored at -20 °C until use. RNA was extracted, cleaned, quantified and purity assessed prior to be transcribed into cDNA. Quantitative Real-Time PCR assay (qPCR) was used to determine transcript abundances for the 28 genes and 5 reference genes (see Table 1 below)

Table1 Abbreviated list of genes quantified by qPCR in the abomasal mucosa of *Ovar-DRB1*0203* carrier and non-carrier lambs following infection with 30000 *T. circumcincta* L3

Functional grouping	Gene names
Cytokine-cytokine receptor signalling	IL1 β , IL2, IL4-6, IL10, IL12, IL13, IL18, IFN γ , TNF α , TGF α , TGF β ₁ , TGF β ₂ , TGF β ₃
Generation of reactive oxygen	Arg2, NOS2A, XDH
Extracellular matrix	MUC2, MUC3A, MUC5AC, TFF2, TFF3, ovar-Gal14, sITLN2, sMCP-1, GATA3
Defensin	MS4A2
Reference genes	GAPDH, ATPase, RPLP0, ACTB, 18s rRNA

A comparison of the mucosal proteome of twin *Ovar-DRB1*0203* carrier and non-carrier lambs undergoing *Teladorsagia circumcincta* infection to identify effector molecules driving resistance.

Four lambs from each genotype were slaughtered before infection (controls), and at 7, and 21 days post infection. Abomasal mucosa scrapings were collected and snap-frozen in liquid nitrogen. The proteome of the samples was analysed and results were confirmed at the mRNA level using quantitative real-time PCR. This study has confirmed the involvement of the *Ovar-DRB1*0203* allele in resistance to *T. circumcincta* in Suffolk sheep. The efficient immune response, particularly the switch from a Th1 to the anti-parasitic Th2 response; both at the cellular and humoral level appear to be a key mechanism underlying this resistance. Additionally, this study shows that the upholding of gastrointestinal integrity, reduced stress-related cell turnover and nutrient utilisation are some of the processes driving resistance due to the *Ovar-DRB1*0203* allele.

3. Main results:

Experiment 1

The *Ovar-DRB1*0203* carrier lambs had significantly lower worm burden, higher mast cell and plasma platelet counts than the *Ovar-DRB1*0203* non-carrier lambs ($P < 0.05$). Before infection, the non-carrier lambs exhibited significantly higher mucosal levels of all antibody isotypes measured compared to the carrier lambs, however, these levels remained relatively stable over the course of infection in the non-carriers while there was a slow buildup of these antibodies in the carriers up to day 21 post infection. The non-carrier lambs had significantly higher plasma lymphocytes, and produced greater contractile force relative to the carrier lambs ($P < 0.05$). There was no significant genotype difference in the levels of plasma eosinophils, monocytes, neutrophils or FEC. This evidence suggests that resistance conferred by *Ovar-DRB1*0203* is acquired rather than innate, depends on worm expulsion rather than fecundity and is dependent on mucosal mast cell proliferation, platelet activation, and IgA and IgE antibody responses. The evidence from this study suggests that the mechanisms underlying nematode resistance conferred by the *Ovar-DRB1*0203* allele, is mediated by mast cell proliferation in the mucosa, and higher IgE and blood platelet levels. Additionally, the lower abomasal worm burden in the carriers, indicate that the low FEC associated with *Ovar-DRB1*0203* allele is due to an effect on worm expulsion rather than on fecundity.

Experiment 2

Analysis of cytokine gene expression profiles in the abomasal mucosa of these lambs showed that the response generated varied through the course of infection and was affected by genotype. The *Ovar-DRB1*0203* carrier lambs had an up-regulation of Th1 cytokines (IL-1 and IFN- γ) on day 3, but this was replaced by an up-regulation of Th2 cytokines (IL-10 and IL-13) by day 7. Conversely, in the *Ovar-DRB1*0203* non-carrier lambs the expression of Th1 and Th2 cytokines was delayed until days 7 and 21, respectively.

Experiment 3

Proteomic analysis of the abomasal mucosa of these lambs, followed by confirmation at the mRNA level revealed the *Ovar-DRB1*0203* carrier lambs to have increased levels of lysozyme-C-3, albumin, and heat shock protein 70, while the *Ovar-DRB1*0203* non-carrier lambs showed increased levels of protein disulfide isomerase A-3, carbonic anhydrase-II, peroxiredoxin-2 and triosephosphate isomerase-1. Lysozyme-C-3 is reported to influence mucus viscosity and transportability, while heat shock protein 70 is known to prolong the survival of stressed cells. Peroxiredoxin-2 is a protein associated with increased oxidative stress and apoptosis, while triosephosphate isomerase-1 is an energy metabolism enzyme. Based on the functions of

these proteins, the carrier lambs seem to limit stress-related cell turnover while the non-carrier lambs respond to energy requirements associated with nematode pathogenesis.

4. Opportunity/Benefit:

This research has advanced our knowledge on the role of the *Ovar-DRB1*0203* allele on resistance to gastrointestinal nematodes in sheep and the biological mechanisms underlying the resistance. It has confirmed the possibility of breeding sheep for increased nematode resistance and established a basis for future research on the mechanisms underlying gastrointestinal nematode resistance.

This study has confirmed the involvement of the *Ovar-DRB1*0203* allele in resistance to *T. circumcincta* in Suffolk sheep. The efficient immune response, particularly the switch from a Th1 to the anti-parasitic Th2 response; both at the cellular and humoral level appear to be a key mechanism underlying this resistance. Additionally, this study shows that the upholding of gastrointestinal integrity, reduced stress-related cell turnover and nutrient utilisation are some of the processes driving resistance due to the *Ovar-DRB1*0203* allele.

In the present study, IgA, and IgE have been shown to play a role in the resistance to gastrointestinal nematodes, however, it is not clear if there exists any functional differences between antibodies from the *Ovar-DRB1*0203* carriers and non-carriers. The opportunity exists to test if there are any difference in the avidity and affinity of IgA and IgE obtained from *Ovar-DRB1*0203* carriers and non-carriers against parasite antigens.

Because of the strong linkage-disequilibrium within the MHC locus, it is difficult to know if the *Ovar-DRB1*0203* is the resistance allele or if it is just associated with a resistance allele, additional studies would be useful to further characterise the role of this allele in nematode resistance. Moreover, how selection based on *Ovar-DRB1*0203* allele may affect resistance to other parasites would need to be explored. Co-infection models could be useful in determining the effect of selecting sheep for increased nematode resistance, using this allele, on other parasites and gastrointestinal nematodes.

5. Dissemination:

International conference

Presented at 22nd International Conference of the World association for the Advancement of Veterinary Parasitology, Calgary, Canada (2009)

National Conferences

Presented at the Irish Society for Parasitology (2008).

Open days

Presented at : Farm Fest 2008 Teagasc Athenry, Sheep 2010 Lyons Estate UCD , Sheep 2012 Teagasc Athenry and Lab open days/visits

Main publications:

Hassan, M., Good, B., Hanrahan, J.P., Campion, D., Sayers, G., Mulcahy, G. and Sweeney, T. (2011). 'The dynamic influence of the DRB1*1101 allele on the resistance of sheep to experimental *Teladorsagia circumcincta* infection'. *Veterinary Research Communications* 42(1); 46

Hassan, M., Hanrahan, J.P., Good, B., Mulcahy, G. and Sweeney, T. (2011). 'A differential interplay between the expression of Th1/Th2/Treg related cytokine genes in *Teladorsagia circumcincta* infected DRB1*1101 carrier lambs'. *Veterinary Research Communications* 42(1); 45

Hassan ML. (2010) The immunogenetics of ovine gastrointestinal nematode resistance. PhD University College Dublin

6. Compiled by: Dr. Barbara Good