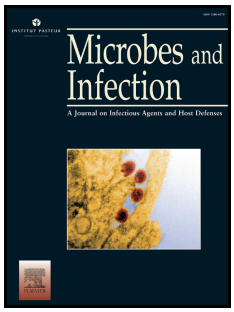
Accepted Manuscript



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PII: S1286-4579(10)00245-5

DOI: 10.1016/j.micinf.2010.09.013

Reference: MICINF 3655

To appear in: *Microbes and Infection*

Received Date: 2 September 2010

Accepted Date: 10 September 2010

Please cite this article as: C. Dold, C.V. Holland. Investigating the underlying mechanism of resistance to Ascaris infection, Microbes and Infection (2010), doi: 10.1016/j.micinf.2010.09.013

Title: Investigating the underlying mechanism of resistance to *Ascaris* infection

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Abstract

The generative mechanism(s) of predisposition to *Ascaris* infection are currently unknown. While many factors play a role in interindividual infection intensity, much focus has been placed on the host's immunological response to infection and the underlying genetics. The present review describes the research conducted that has examined various immunological parameters and genetic factors that may play a role in resistance to ascariasis. We also discuss the contribution that animal models have made to our understanding of resistance to the parasitic roundworm and their role in possible future work.

ASCARIS; PREDISPOSITION; RESISTANCE; SUSCEPTIBILITY; ANIMAL MODELS

Introduction

Predisposition to *Ascaris* infection was first noted in the 1980s and since then has attracted the attention of parasitologists interested in attempting to uncover the underlying mechanism of host resistance. There are several factors that contribute to intraspecific variation to *Ascaris* infection, which were broadly and briefly discussed already in the present issue (Dold and Holland, current issue). Exposure to a pathogen is regulated by ecological and behavioural factors [1], and studies investigating behavioural-mediated acquisition of infection were noted in the previous review. Furthermore, the ability of hosts to regulate parasites and thus control resistance or susceptibility at the individual and population level, through immune responses is a very important determinant of host variation in susceptibility to helminth infection. Differing immune responses to infection, either innate or acquired, are ultimately determined by host genetics. Therefore, recent emphasis has been placed on investigating various immunological parameters and genetic factors as possible contributors to resistance to heavy *Ascaris* burdens. In this review, we discuss work undertaken investigating the immunological and genetic basis of differing *Ascaris* worm burdens in both humans and other animal models.

Animal models of *Ascaris* infection

Studies of *A. lumbricoides* worms in the human host are limited due to ethical considerations. Therefore, research involving human hosts is confined to the adult stage of *Ascaris* infection. Furthermore, difficulties exist in determining the complex immunoepidemiological patterns or importance of host genetics in disease development under field conditions, due to the

multiplicity of environmental factors which must be considered [2, 3]. Model organisms facilitate a greater degree of control over the different parameters under study, since the ecological parameters that impact helminth infection can be controlled or eliminated [4]. For these reasons, many researchers have turned to exploring *Ascaris* infection in a wide variety of model organisms, which has contributed to our current knowledge of both the migratory pattern and impact of larvae during early infection as well as the immune response to *Ascaris* spp.

As discussed by Boes and Helwigh [4], an appropriate model of helminth infection should mimic the human host, the parasite and the human host parasite system. *A. suum* infection in the pig is a naturally occurring host-parasite system and *A. suum* is closely related to the human parasite species, *A. lumbricoides* [e.g. 5]. Therefore, the pig serves as a suitable model for human *Ascaris* infection.

The mouse has been extensively studied as a model of early *Ascaris* infection. Infection in mice only represents larval stages of the life cycle as worms do not return to the small intestine to mature, therefore this constitutes an abnormal host-parasite relationship. However, Slotved *et al.* [6] confirmed by means of comparative work with the pig that the migratory pattern of *A. suum* is similar in murine and porcine hosts. Therefore, the mouse is deemed an appropriate host of early *Ascaris* infection [6].

The immunological response to *Ascaris* infection

While resistance to helminth infection tends to be associated with Th2 responses, the precise underlying immunological mechanisms that lead to the expulsion of *Ascaris* worms are yet to be defined.

Various surveys of endemic host antibody and cellular immune response patterns to antigens have been undertaken, typically prior to and after chemotherapy. Such studies generally correlate the immune signalling or effector molecules with infection intensity and/or more rarely predisposition.

Early field surveys examined the relationship between specific immunoglobulin responses in *A. lumbricoides*-infected humans and intensity of infection. While the magnitude of the antibody response is likely to be determined by age, infection intensity, history of infection and individual host genetics, studies have shown that antibodies of all isotypes (IgM, IgG, IgA and IgE) are induced during human infections with *A. lumbricoides* (Cooper *et al.*, 2000). Antibody responses appear to be stimulated by *Ascaris* larvae as demonstrated by studies involving experimental infection whereby antibodies were detected shortly after infection, lasting 3-4 months p.i. [7]. In studies where interruption of transmission during the winter has been documented, host antibody levels coincide with parasite transmission in the remaining months [7].

The age-related changes in intensity patterns of *A. lumbricoides* infections observed in human populations are mirrored by the antibody response levels recorded, indicating that the humoral immune response is dependent on burden as opposed to being protective [8, 9]. Coupled with this, in studies conducted in Bangladesh and Ecuador, Palmer *et al.* [10] and Cooper *et al.* [11] both demonstrated that antibody levels are reflective of parasite burdens.

Contrasting IgG and IgE responses have been detected in lightly- and heavily-infected hosts. Geiger *et al.* [12] recorded that both total IgG and the subclass IgG4 were significantly elevated in response to *A. lumbricoides* in infected patient groups. In contrast to this, a minimal IgG4 response was detected in infected hosts in a Nigerian population [13], while Cooper *et al.* [11] also showed that levels of *Ascaris*-specific IgG, IgG1 and IgG2 antibodies are positively correlated with parasite burdens. High circulating levels of IgE, in excess of 10,000 IU/ml [14], and *Ascaris*-specific IgE have been consistently associated with human ascariasis [14-18]. Heightened levels of IgE in infected hosts has been considered to be a result of direct mitogenic effects of *Ascaris* allergens on B cells responsive to *Ascaris* allergens [19], as many allergens are secreted during larval migration [20, 21] or are associated with the production of large amounts of IL-4 [11]. A negative association between IgE and susceptibility to *A. lumbricoides* reinfection was reported in a Venezuelan population [17], and McSharry *et al.* [13] found that levels of specific and total IgE were related to protection rather than to exposure to infection, indicating that high IgE producers have natural immunity to *A. lumbricoides* infection.

Focus has also been placed on cytokine expression and *A. lumbricoides* infection patterns in human populations. Cooper and colleagues [11] first described the cytokine phenotypes associated with *A. lumbricoides* infection in endemic Ecuadorian populations and thus demonstrated that the cellular immune response to *Ascaris* antigens is characterised by a highly polarised Th2 cytokine response. In this study, frequencies of peripheral blood mononuclear cells, expressing IL-4 and IL-5 were significantly greater in the infected group, whereas the frequencies of IL-10 and IFN-γ were similar in the both the infected and control groups. While levels of IFN-γ were not raised in infected subjects, there was no evidence for impaired IFN-γ expression, which may indicate a mixed Th1/Th2 response [11].

A study on a Cameroonian population further highlighted the importance of a Th2 response and resistance to *Ascaris* infection, as hosts considered persistently susceptible were characterised by a weak Th2 response [22]. Resistance to reinfection at 8-9 months post-chemotherapy was positively related to pre-treatment levels of IL-5 [22], indicating a negative association of this Th2 cytokine with general susceptibility. This is consistent with the effectiveness of IL-5 driven Th2 cellular responses being greatest against larvae, rather than established adult infections [23]. Furthermore, IL-13 levels have been documented to be negatively associated with general susceptibility [22]. In addition a separate study recorded increased IL-13 levels in the older age classes, in which lower infection intensities were observed [24].

Contrasting levels of IL-10 have been documented in endemic populations, with some studies showing no difference in expression between the infected and uninfected groups [11], while

other authors document an inverse correlation with intensity of *A. lumbricoides* infection [24]. It has been speculated by Turner *et al.* [24] that the presence of IL-10 may be "reflective of a regulatory network that is induced to hold potentially damaging inflammatory responses in check as has been proposed by the hygiene hypothesis". An example of the inflammatory responses to *Ascaris* infection was marked by increased levels of C-reactive protein, ferritin and eosinophil cationic protein detected by McSharry and colleagues [13] in Nigerian children. However, heightened levels were detected in putatively immune hosts, demonstrating that inflammation may represent an antiparasite effector mechanism.

The studies detailed above which evaluated cytokine responses to *Ascaris* infection all revealed that Th2 cytokines are important in mediating resistance to *Ascaris* infection [14, 22, 24], although the way in which the Th2 response coordinates this protection is still unknown. Both Turner *et al* [24] and Jackson *et al.* [22] conducted their studies in Cameroon albeit in different provinces. Despite this, when results from the two studies were compared, variations in the Th2 cytokines were observed with different age-dependent trends at different localities. This led the authors to conclude that age- and location-related differences may impact upon cytokine responses to infection, adding another layer of complexity to the immunological response to *A. lumbricoides* and an additional factor to consider when implementing control strategies.

Studies on the immunology of *Ascaris* infection in the pig model have contributed to our understanding of the response in human hosts. Various immunological studies in the pig have

focused on the possibility of stimulating a pre-hepatic barrier. Reduced recovery of hepatic larvae and associated inflammation indicating protective intestinal immunity has been observed in naturally-infected pigs [25-27]. Furthermore, on return to the small intestine post migration, pre-adult larvae shift distally and are expelled between days 14-21 p.i. This pattern of self-cure expulsion was observed during experimental single infections and did not appear to be influenced by the size of the inoculation dose [28].

It has been speculated that expulsion of larvae on return to the intestine is a result of either a density-dependent self-reduction of the larval population or a specific immune-mediated reaction. Eriksen [29] noted that, during primary infections, increased mucus secretion and desquamation of the intestinal epithelial cells occurs concurrently with the re-arrival of larvae to the intestinal lumen. Once larvae left the intestine, an increase in mast cells was observed and normal tissue architecture was rapidly re-established. During secondary infections, the nature of the reaction in the intestine and all migratory route tissues was similar yet heightened and was observed earlier. Miquel *et al.* [30] examined the dynamics of the immune responses before, during and after expulsion. An increase in *A. suum*-specific IgM and IgA antibody-secreting cells was found over the course of expulsion period, with a more pronounced increase in IgA levels. The antibody response to *Ascaris* antigens in the luminal small intestine was also more prominent in the proximal than in the distal jejunum, which may be related to the longer antigen exposure time at this site, as large numbers remain at the former site for 5-7 days prior to expulsion.

While it is known that the development of strong immunoglobulin E (IgE) is characteristic of helminth infections, the lack of porcine anti-IgE prohibits studies on the dynamics of the serum IgE during helminth infection in porcine hosts. However, biological activities related to IgE production, such as degranulation of intestinal mucosal mast cells, have been observed in trickle-infected pigs [25, 31]. However, this response was not observed in a later single infection experiment [30], indicating that repeated exposure is necessary. Increased serum levels of IgA and IgG have been detected in response to antigens in infected pigs [32-35], and Frontera *et al.* [34] also demonstrated that IgG was positively correlated with the severity of the hepatic inflammatory reaction but negatively correlated with the number of larvae recovered in the lungs, indicating a protective function.

The characteristic hepatic inflammatory reaction in pigs, known as white spot formation have been proposed to play a role in immunity to *A. suum* infection in pigs [36, 37]. *A. suum* larval debris has been previously detected within GT-WS [38], centrally located within the granular mass [38], while antigenic deposits thought to be remains of L_3 larvae have been trapped by hepatic inflammatory cells [39]. Numerous macrophages, some fibroblasts and lymphocytes have been observed in the granulomatous lesions. In some cases, Peréz *et al.* [40] reported that larger granulomas were surrounded by fibrous connective tissue. The diffuse lymphocyte infiltrate of granulomatous lesions was composed mainly of CD3+ T cells, with only a few CD79a+ B cells. The older lymphoid follicles were mainly composed of CD79a+ B cells with IgG+ plasma cells at the periphery of the lesions, indicating that this immunoglobulin is important in the local response to *Ascaris* hepatic tissue invasion. IgA+ and IgM+ plasma cells were rare.

Anti-MHC class II mAb reacted with the majority of the macrophages and lymphocytes in granulomatous lesions, but this cannot be interpreted as activation as this antigen is constitutively expressed by porcine T lymphocytes [40].

The larvae-induced GT-WS have been proposed as a precursor to the lymphonodular types WS (LN-WS) [36, 41], as the appearance of the latter on day 10 p.i. in pigs coincides with the healing of GT-WS [28]. Peréz *et al.* [40] likened the LN-WS cellular distribution to that of the cortex of lymph nodes, with the majority of lymphoid cells being $CD79a^+$ B cells with a few $CD3^+$ T cells. A variable number of the B cells were shown to be IgG+ plasma cells, while only a few IgM^+ and IgA^+ cells were detected. Dendritic-like cells with cytoplasmic projections were also recorded, which may be responsible for enhancing antigen presentation.

Increased liver pathology has been shown to be related to a reduction in lung larval numbers in repeated experimental inoculations [26, 29, 42] and in consistently naturally-exposed herds [26]. Studies in which pre-hepatic protective immunity was not observed involved immunization with ultraviolet-irradiated *A. suum* eggs [43] or with different antigen preparations [33, 44]. Furthermore, circumvention of the hepatic stages of migration may decrease intestinal expulsion [45] indicating a possible immunoregulatory role of the liver in initiating larval expulsion on return to the small intestine.

Eosinophil infiltrates are typically observed during the course of *A. suum* infection in pigs, and levels of peripheral blood eosinophilia are elevated in secondary infections [29]. A localised

response of eosinophils around larvae may be reflective of the extent of larval invasion rather than necessarily being involved in overall pathology. Eosinophils have been observed in histopathological sections of liver and lung tissue in pigs and also mice infected with *Ascaris* [29, 40, 46, 47] yet it is not clear if these cells were present at the time of parasite killing or a consequence of larval death and tissue damage.

The inflammatory reaction in response to invading *Ascaris* larvae has been examined previously in model organisms such as calves [48], guinea pigs [49, 50], rabbits [51] and mice [29, 46]. Eriksen [29] demonstrated that WS in mice were detected in the liver tissue and omentum, some of which contained immobilised larvae. This reaction, however, was less marked in athymic mice, indicating that WS were influenced by a thymus-dependent immune response as was also later demonstrated in pigs (described earlier) [40]. Crandall *et al.* [52] have examined the quantitative changes in the splenocyte populations in *A. suum*-infected C57BL/6j and reported an increase in lymphocytes, eosinophils, neutrophils and plasma cells at 11 days p.i. Coupled with this, an increase in IgG and IgM levels was observed in the third week of infection. Further work on the humoral response to *Ascaris* infection was undertaken in guinea pigs, whereby protection (significantly reduced larval numbers) was observed in recipient animals once administered IgG1+IgE or IgG2 [53].

Experiments on guinea pigs also involved donating cells drained from the hepatic, mediastinal and mesenteric nodes of animals demonstrating protection and transplanting them to recipient animals, which were then infected once. Cells from the hepatic nodes were shown to be

significantly more effective in conferring protective immunity upon normal recipients. Therefore Khoury *et al.* [53] concluded that "the major protective immune mechanisms to *A. suum* take place at the hepatic level".

Most recently, focus has been placed on the liver while investigating the mechanism of resistance in a mouse model of early infection developed by Lewis *et al.* [54]. Reduced larval burdens are consistently observed in the resistant CBA/Ca mice even though similar hepatic burdens are observed in this mouse strain and the susceptible C57BL/6j strain [47, 55]. Combined with this, comparative histopathology of the lung and BAL fluid assessment indicated that the pulmonary response in both strains was more reflective of burden rather than being protective [47]. Therefore, it was concluded that a hepatic or post-hepatic factor that varies between the two mouse strains may account for the divergence in lung larval burdens and thus resistance/susceptibility. On comparing the inflammatory cells stained in histopathological sections of liver tissue, it was later noted that resistant CBA/Ca mice respond earlier and at a time that coincides with the reduction of larvae while a similar response was not observed in C57BL/6j mice until the larvae has successfully migrated to the lungs [55].

Dissecting genetic control of resistance to helminth infection

While the generative mechanisms of predisposition are currently unknown, it is believed that host genetics may play a major role [56-61]. Familial patterning to *Ascaris* infection has been

noted in several studies (Chai *et al.*, 1983; Forrester *et al.*, 1990; Chan *et al.*, 1994a). However, familial aggregation does not prove genetic control. Dissecting the genetic control of resistance to helminth infection is problematic for various reasons. Studies of familial aggregation in human host populations are confounded by environmental, sanitation and family behavioural factors, which are difficult to disentangle from the effects of host genetics. Furthermore, the complexity of the host-parasite relationship and multiple possible host immune responses indicates that the genetic factors influencing the host's capacity to respond protectively to infection are polygenic, specified by many minor susceptibility genes rather than primarily by a few major loci [62].

As reviewed by Quinnell [63], susceptibility to helminth infection in human populations has been shown to vary among ethnic groups, yet reliable studies investigating the effect of genetic relatedness on helminth infections requires pedigree studies. In a pedigree study, one can trace a variant phenotype in related individuals living in different households and unrelated individuals living in the same household, therefore eliminating environmental factors that may mask genetic effects on the phenotype.

Detailed research has been conducted in East Nepal [56, 60, 61] using genome scanning of individual human hosts from a Jirel population (Jiri Helminth Project). The worm burden of the individuals in the study group was first assessed to determine the intensity level of infection. The study design, which utilizes a single large pedigree, allows for discrimination between the effects of shared environment and genetics. The initial research focused on clarifying whether

host genetics in fact played a role in predisposition. High within-individual correlations indicated that there is individual predisposition, which also appeared to be heritable. From this data it was calculated that a host genetic component accounted for 30-50% of variation in the worm burden harboured, while the effects of a shared environment accounted for 3-13% of variation in helminth load [60]. A similar heritability measure (0.35 for total *A. suum* eggs excretion and 0.44 for actual worm counts) was later identified in pigs [64].

Conducting further research on the Jiri pedigree, Williams-Blangero *et al.* [56] undertook a genome scan project in which they found strong evidence for two distinct quantitative trait loci (QTL) influencing the susceptibility to *Ascaris* infection. The genes are on chromosomes 1 and 13 and there was also suggestive evidence for a third locus influencing *Ascaris* burden on chromosome 8. Evidence for the latter quantitative trait locus was significantly less than that for the former two. Further genome scanning in a higher number of host individuals of the Jirel pedigree was subsequently undertaken, in which six and three chromosomal regions were identified respectively, with evidence for QTLs influencing the intensity of and susceptibility to *Ascaris* infection [61]. Within the QTLs, multiple immune-related genes have been identified. For example, Williams-Blangero *et al.* [56, 61] indicated that the gene *TNFSF13B* (also known as *BlyS*) is involved in determining susceptibility and resistance to *A. lumbricoides* infection.

As discussed by Shaw and Quinnell [65], “the similarity between immune responses in asthma and helminth infection has led to the hypothesis that alleles predisposing to asthma provide resistance to helminth infection.” Recently, the relationship between three genes (LIG4**,**

TNFSF13B and IRS2) and the IgE and IgG response to *Ascaris* infection and a resistance marker, the ABA-1 allergen was investigated in an endemic Columbian population [66]. Coupled with this, a case-control study was conducted to investigate whether observed polymorphisms were associated with predisposition to asthma and atopy. In this study, two of the assessed genes' genomic location (13q33.3) was implicated in resistance to *Ascaris* infection [56, 61]. The two genes TNFSF13B and LIG4 were shown to modulate the IgE and IgG response to *A. lumbricoides* infection, thus appearing to be involved in conferring protective immunity to the helminth parasite.

Investigating the association of host candidate genes with resistance is generally based on previous genetic studies or is suggested on the basis of immunological grounds [63]. Since the MHC gene products are involved in regulation of T-cell function, there has been much focus on investigating the involvement of MHC-linked genes coupled with genes for cytokine and chemokine receptors in controlling resistance to helminth infection [67].

The link between MHC genes and the immune response stimulated research on the association of MHC antigens and resistance to infection. Holland *et al.* [58] undertook an associational study in which the distribution of human leukocyte antigen HLA-A, HLA-B and HLA-C was assessed in consistently uninfected, lightly infected and heavily infected Nigerian children. The frequency of occurrence the A30/31 antigen was significantly higher in the hosts who were consistently infected, indicating a role of the MHC in determining resistance to *Ascaris* infection. Further to this, relationships of the candidate genes, the non-MHC gene signal

transducer and activator of transcription 6 (STAT 6) which promotes expression of Th2 associated genes have been shown to be linked with *Ascaris* resistance [68]. Furthermore, polymorphisms in the β_2-adrenoreceptor gene were found to correlate with varying *Ascaris* egg loads in Venezuelan children [69]. The latter candidate study is of particular interest as one of the polymorphisms observed accounted for 25% of the variation in the *Ascaris* egg loads, and also the gene is located on the same chromosome 5q region as the SMI gene, which is known to influence variation in schistosome infection [70, 71]. While work with a single candidate gene does not reveal the polygenic control of resistance to helminth infection, it may show evidence for a major causative gene of host variation.

The ability to manipulate the genetic background of inbred mice is beneficial to studies in which the association between candidate genes and a particular trait of interest are being studied. Furthermore, as stated by Dietrich (2001), studying mouse genetics could lead to the identification of human homologs which are likely to affect the outcome of infections in man. Neither genome scans nor candidate gene studies have been conducted in mice infected with *Ascaris*. Contrasting host intensity phenotypes in C57BL/6j and CBA/Ca mice provides a model in which the association between candidate genes and *Ascaris* infection intensity can be investigated. Recently, the association between the Intelectin-2 gene and resistance was examined in F_2 mice derived from the two mouse strains [72]. The candidate gene is this particular study was selected based on its dominant expression in resistant mice in various models of helminth infection [73-75] combined with its absence in the susceptible C57BL/6j

mouse strain [75]. Despite these previous findings, Intelectin-2 gene copy number was not significantly linked to larval burden or resistance [72].

Resistance to helminthic infection is multifactorial but a key question posed in a review by Quinnell (2003) is whether the same genes are involved in controlling different helminth species. Therefore, while Intelectin-2 did not provide a fruitful line of enquiry, immune response genes, particularly those shown to play a role in resistance to other parasitic infections are likely candidates for future studies.

Future directions and considerations

Even though the mechanistic basis of resistance to *Ascaris* infection is yet to be discovered, the research conducted has provided an insight and direction for future work. Previous studies have highlighted various considerations while designing further research in predisposition

Many immunological studies involve assessing the relationship between infection intensity and *Ascaris* antigens. Antigens described for *A. suum* and *A. lumbricoides* have been shown to be both stage- and species specific [76, 77]. However, ABA-1 is the most abundant protein in the parasite's body fluid [78] is present in all stages of the *Ascaris* lifecycle [76, 77] and has a highly conserved amino acid sequence between the two species [79]. McSharry and colleagues [13] demonstrated that when three *A. lumbricoides* antigens (body/pseudocoelomic fluid, a

commercially prepared crude allergen known as *Ascaris* p1 and ABA-1) were assessed, only the refined ABA-1 antigen provided evidence for a significant relationship between predisposition status and parasite-specific IgE. While it is now accepted that *Ascaris* spp. hatch as L_3 stage larvae [80, 81], previous studies have demonstrated a low response to L_2/L_3 ES antigen significantly correlated with high worm burdens in the intestine [32]. This, in combination with the differing results obtained by McSharry *et al.* [13] dependent on the target antigen assessed, indicates that antigen response studies would benefit from the use of standard antigens. Many of these antigens have been characterised but yet few studies in humans and pigs involving parenteral application have induced a protective response in the host comparable to that induced by egg inoculation.

Multiple locations involved in the migratory path have been presented as sites at which the mechanism of resistance is manifested. As highlighted in this review, experiments in pigs and rodents have provided evidence for a pre-hepatic barrier, immobilization of larvae in the liver and expulsion of worms from the intestine post migration, the latter of which is influenced by the earlier invasion of the liver. Further direct experimental work is required on the local response in order to elucidate the mechanisms underlying the observed pre-hepatic and hepatic barriers. Coupled with the inability to conduct direct experiments on humans, immunological studies are also constrained to assessing peripheral responses. Jungersen *et al.* [82] recognised the importance of assessing the immunological response to *A. suum* infection at the site at which immunity is manifested. The lymphocyte population which recognised antigens was dependent on the draining area of the lymph nodes assessed [82], which may be a

result of the stage-specific antigens [76] the surrounding organs were exposed to during infection or may be dependent on the effector mechanisms of the immune response in the particular organ. For obvious ethical reasons, assessment of local hepatic and intestinal responses to early infection is more achievable in model organisms.

Despite the large number of studies presenting evidence of a role for the liver in resistance to *Ascaris* infection, the experiments were undertaken in different model organisms, in some of which resistance/susceptibility was not established. Therefore, the optimised and robust mouse model of early *Ascaris* infection, mentioned above now provides an attractive system for economical and practical future work in which the effective hepatic response observed in CBA/Ca mice can be further dissected. To date the most detailed study undertaken in which the cells involved in the hepatic response to larval invasion were characterised has been conducted in pigs by Peréz *et al.* [40]. Future work in which the immunoregulatory cell population of infected resistant and susceptible mouse livers will greatly add to our knowledge of possible hepatic anti-*Ascaris* effector mechanisms.

In contrast with previous work in which exposure to infection appeared to mask the influence of host genetic factors on predisposition [83], Williams-Blangero *et al.* [56, 60, 61] clearly demonstrated a role for host genetics in determining infection intensity. In their most recent paper, Williams-Blangero and colleagues concluded that efforts will now focus on the characterisation of the specific genes underlying the three significant QTL regions that have been identified [61]. The importance of this work was recently highlighted, as identifying the

underlying genes could provide new approaches to the treatment or prevention of infection [84]. As discussed, the relationship between candidate genes and *Ascaris* infection have been conducted in both human and rodent populations and therefore the information gained from the research conducted on the Jirel pedigree population may be furthered both within the pedigree and established animal models. This possible combined approach would facilitate high throughput studies in which the multigenic mechanisms of resistance could be studied.

Research undertaken with the Jirel pedigree population focuses solely on host genes whereas other parasitologists have become focused on parasite genes and their influence on parasite distribution within a population. It is known that parasite genes determine characters such as virulence and vary in their capacity to induce host responses (immunogenicity). For example, Nejsum *et al.* [85] conducted experimental pig infections with equal proportions of *A. suum* worms with unique mtDNA haplotypes and documented an uneven distribution of the four different genotypes in the intestine, which increased over the course of infection. Coupled with this, the spatial distribution of the four haplotypes also varied across the host gut length. Therefore, it appears that parasite genetics require further attention in host susceptibility studies especially when comparing results from different regions, in which various haplotypes would be prominent.

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