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Role of the bovine immune system and genome in resistance to gastrointestinal nematodes

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Abstract

Gastrointestinal nematode infections of cattle remain a constraint on the efficient raising of cattle on pasture throughout the world. Most of the common genera of parasites found in cattle stimulate an effective level of protective immunity in most animals within the herd after the animals have been on pasture for several months. In contrast, cattle remain susceptible to infection by *Ostertagia* for many months, and immunity that actually reduces the development of newly acquired larvae is usually not evident until the animals are more than 2 years old. This prolonged susceptibility to reinfection is a major reason that this parasite remains the most economically important GI nematode in temperate regions of the world. Although, animals remain susceptible to reinfection for a prolonged period of time, there are a number of manifestations of the immune response that result in an enhanced level of herd immunity. These include a delay in the development time of the parasites, an increase in the number of larvae that undergo an inhibition in development, morphological changes in the worms, stunting of newly acquired worms, and most importantly a reduction in the number of eggs produced by the female worms. The overall result of these manifestations of immunity is a reduction in parasite transmission within the cattle herd.

The immune mechanisms responsible for these different types of functional immunity remain to be defined. In general, GI nematode infections in mammals elicit very strong Th2-like responses characterized by high levels of Interleukin 4 (IL4), high levels of IgG1 and IgE antibodies, and large numbers of mast cells. In cattle, the most extensively studied GI nematode, in regards to host immune responses, is *Ostertagia ostertagi*. In *Ostertagia* infections, antigens are presented to the host in the draining lymph nodes very soon after infection, and within the first 3–4 days of infection these cells have left the nodes, entered the peripheral circulation, and have homed to tissues immediately surrounding the parasite where they become established. The immune response seen in the abomasum is in many ways are similar to that seen other mammalian hosts, with high

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levels of expression of IL4 in the draining lymph nodes and in lymphocytes isolated from the mucosa. But unlike a number of other systems, lymphocyte populations taken from *Ostertagia* infected cattle seem to be up-regulated for a number of other cytokines, most notably Interferon-(IFN-(), implying that in *Ostertagia* infections, the immune response elicit is not simply a stereotypic Th2 response. In addition, effector cell populations in the tissues surrounding the parasites, are not typical, inferring the *Ostertagia* has evolved means to suppress or evade protective immune mechanisms.

Studies have also demonstrated that the number of nematode eggs/gram (EPG) in feces of pastured cattle is strongly influenced by host genetics and that the heritability of this trait is approxiamately 0.30. In addition, EPG values are not ⟨⟨normally⟩⟩ distributed and a small percentage of a herd is responsible for the majority of parasite transmission. This suggests that genetic management of a small percentage of the herd can considerably reduce overall parasite transmission. A selective breeding program has been initiated to identify the host genes controlling resistance/susceptibility to the parasites. The best indicator of the number of *Cooperia* infecting a host is the EPG value, while *Ostertagia* is best measured by serum pepsinogen levels, weight gain, and measures of anemia. Other phenotypic measures are either not significantly associated with parasite numbers or are very weakly correlated.

In addition, calves can be separated into three types: (1) Type I which never demonstrates high EPG values, (2) Type II which shows rises in EPG values through the first 2 months on pasture which then fall and remain at levels associated with Type I calves, and (3) Type III calves which maintain high EPG levels. The approximate percentage of these calves is 25:50:25 respectively. Because these cattle are segregating for traits involved in resistance and susceptibility to GI nematodes, this resource population is being used to effectively detect the genomic locations of these Economic Trait Loci (ETL). For relational analysis between phenotype and genome location, over 80,000 genotypes have been generated by PCR amplification, and marker genotypes have been scored to produce inheritance data. The marker allele inheritance data is currently being statistically analyzed to detect patterns of co-segregation between allele haplotype and EPG phenotypes. Statistical power of this genome-wide scan has been strengthened by including genotypic data from the historic pedigree. In our herd, paternal half-sib families range from 5–13 progeny/sire, and extensive marker genotypes are available from ancestors of the population most of which are paternally descended from a single founding sire.

Once ETL have been identified the next will be to refine ETL map resolution in attempt to discover the genes underlying disease phenotypes. Accurate identification of genes controlling resistance will offer the producer several alternatives for disease control. For a non-organic producer, the small percentage of susceptible animals can be targeted for drug administration. This approach would reduce both the cost of anthelmintics used and the odds for selection of drug resistant mutants, because the selective agent (drug) would not be applied over the entire parasite population. A second treatment option would be based on correcting a heritable immunologic condition. In this case, susceptible animals could be the targets for immunotherapy involving vaccines of immunomodulation. A final option would be genetic selection to remove susceptible animals from the herd. Producers with a high degree of risk for parasite-induced production losses, such as organic producers of producers in geographic areas with environmental conditions favorable to high rates of transmission would benefit the most from this strategy. In contrast, producers at low risk could take a more conservative approach and select against susceptibility when other factors were equal. Published by Elsevier Science B.V.

Keywords: Cattle; Bovine; Immunity; Interleukin; Resistance; Genomics; Genetics; Economic trait locus; ETL; Gene mapping; Functional genomics; Nematodes; Helminths

1. Introduction

Gastrointestinal nematode infections of cattle remain a constraint on the efficient raising of cattle on pasture throughout the world. In less developed agricultural systems the severity of disease caused by these parasites may approach the classical clinical signs of stunted growth, tissue edema, and severe diarrhea. In more affluent agricultural systems the extensive use of highly efficacious broad spectrum anthelmintics has resulted in a situation where clinical disease is not commonly encountered. But even in these intensively managed herds, the parasites hinder optimal growth and productivity of their hosts. Numerous studies have shown that even in well-managed herds with no signs of clinical parasitism, the presence of the parasites in the herds results in decreased growth in young animals (Hawkins, 1993; Ploeger et al., 1990), and decreased milk production by adult cows (Thomas et al., 1984; Gross et al., 1999). This continued economic impact by the parasites has resulted in a situation where the drugs are being used more and more frequently, and/or in drug formulations where anthelmintic activity persists for long periods of time. At the same time current strategic parasite control programs call for the treatment of a large percentage or in some cases all members of the host population. In addition, the new endectocides which are also active against a number of ectoparasites are being used more and more heavily for control of a broad range of parasites. Because the optimal treatment times for endo- and ecto-parasites is often not the same, there is a resulting increase in overall drug usage in order to achieve desired levels of efficacy. As a result, modern control parasite programs are a significant production cost which lowers overall producer efficiency and profitability. An unwanted result of this increased drug usage has been the wide-spread appearance of anthelmintic-resistant parasites in small ruminants throughout the world (Waller, 1977), and the documented appearance of anthelmintic resistance in cattle nematodes in New Zealand (Vermunt et al., 1995; Hosking et al., 1996) and Great Britain (Stafford and Coles, 1999).

In addition to the high cost and consequences of over use, management programs based solely on anthelmintic administration are coming under increased scrutiny by consumers and producers who are concerned by the quantity and effects of man-made chemicals both environmentally and in the food supply. These concerns have led to a growing number of producers adopting “more sustainable” or “organic” husbandry programs, which look to reduce or eliminate the application of man-made chemicals to their herds. For these producers, GI nematodes are arguably the most economically important disease causing organisms affecting their management systems. For producers who cannot afford, or do not want to use modern anthelmintics control options are few. The parasites can be controlled by pasture management programs, but these are often complex and/or do not make optimal use of the pasture resources. Attempts to use biological control agents such as nematophagous fungi have yielded promising laboratory results (Gronvold et al., 1993; Larsen et al., 1997), but have not yet been commercially feasible.

An increasingly attractive adjunct or alternative for control of GI nematodes in cattle is the identification of host genes that influence acquired or innate resistance to the parasites and the use of the vast potential of the host genome to reduce parasite transmission in cattle populations. Because of the nature of the disease caused by these parasites, control of disease does not require absolute protection from infection. Instead an optimal control

program should minimize both the impact of the parasites on productivity and the level of economic input into the production system, while at the same time maximizing utilization of renewable resources such as pastures.

2. Immunity to GI nematodes of cattle

Immunity to infection with GI nematodes in cattle can be manifested in a number of ways. Most of the common genera of parasites found in cattle stimulate an effective level of protective immunity in most animals within the herd after the animals have been on pasture for several months. Reinfection with these parasites results in a significant reduction in the number of worms that become established in the grazing animals. Parasites such as *Dictyocaulus viviparous* and *Oesophagostomum radiatum* are extremely effective in eliciting strong protective immune responses. A primary exposure of previously naive animals to infection or even to parasite antigens results in a very significant reduction in the number of parasites that can become established after a subsequent infection (Rubin and Lucker, 1956; Weber and Lucker, 1959; Gasbarre and Canals, 1989). As a result these parasites remain largely a problem for only the youngest animals in the herd. Other parasites such as *Cooperia* sp. and *Haemonchus placei* require a longer period of exposure before this level of protective immunity is seen, but even with these parasites, calves at the end of their first grazing season will significantly reduce the number of incoming larvae that can successfully become established. In contrast, cattle remain susceptible to infection by *Ostertagia* for many months, and immunity that actually reduces the development of newly acquired larvae is usually not evident until the animals are more than 2 years old. This prolonged susceptibility to reinfection is a major reason that this parasite remains the most economically important GI nematode in temperate regions of the world. Although, animals may remain susceptible to reinfection for a prolonged period of time, there are a number of manifestations of the immune response that result in an enhanced level of herd immunity. These include a delay in the development time of the parasites, an increase in the number of larvae that inhibit, morphological changes in the worms, stunting of newly acquired worms, and most importantly a reduction in the number of eggs produced by the female worms (Michel, 1963, 1967; Michel et al., 1972). Eventually, even in *Ostertagia* infections immune responses that reduce the number of parasites developing after subsequent infection appear (Michel, 1963, 1970; Michel et al., 1973). The overall result of all these manifestations of the immune response is a reduction in parasite transmission within the cattle herd.

In cattle, different parasite species may or may not be susceptible to the same types of immune responses. Schmidt et al. (1998) did not find differences in the diversity of parasite genera in calves from different Aberdeen Angus sires, and concluded that the host has limited effect on diversity of parasite genera. This implies that resistance to the different genera of parasites is similar and that resistant individuals will be resistant across the spectra of parasite species. In contrast, in studies in our laboratory using naive calves placed upon infected pastures for different periods of time, the numbers of *Ostertagia* and *Cooperia* were significantly correlated after 1 month on pasture, but after a period of 4–5 months there was no significant correlation between the numbers of these two parasites in individual animals

Table 1
Correlation between the numbers of *O. ostertagi* and *C. oncophora* recovered from previously naive calves after grazing infected pastures for different periods of time

Time on pasture	*R* value (Number of *Ostertagia* Number of *Cooperia*)	*P* value
1 month ($n = 14$)	0.649	0.012
4–5 months ($n = 39$)	0.133	0.419

(Table 1). This lack of correlation indicates that immunity to the two parasites is not the same in individuals in the herd, and that different immune mechanisms may be important in resistance to different parasite species.

3. Interaction of GI nematodes and the immune system

The immune mechanisms responsible for these different types of functional immunity remain to be defined. Many assumptions concerning the immunological mechanisms involved are taken from rodent model systems. All infections tend to preferentially stimulate one of two types of mutually antagonistic immune responses. These responses referred to as Th1- or Th2-like arise as the result of the stimulation of different subsets of T-helper lymphocytes. The result of stimulation of either of these subsets is the elaboration and secretion of a wide range of cellular communicators termed cytokines. Each of these cytokines exhibits very specific effects on all cell types bearing a surface receptor for the given cytokine. Cytokines can exhibit a variety of effects on different cell types, ranging from stimulation to inhibition. In addition, the cytokine network is highly regulated and has many redundancies, i.e. a number of different cytokines can have the same effect on the same cell type, and many even share the same receptor. It is clear that GI nematode infections in mammals elicit a very strong Th2-like response (Svetic et al., 1993), which is characterized by high levels of the cytokine Interleukin 4 (IL4), high levels of IgG1 and IgE antibodies, and large numbers of mast cells. In mice, it has been repeatedly demonstrated that IL4 is directly involved in protective immunity to intestinal nematodes (Urban et al., 1991, 1992, 1995). But it is also evident that the protective responses are complex, and there are no single dominant effector mechanisms (Else and Finkelman, 1998).

In cattle, the most extensively studied GI nematode, in regards to host immune responses, is *Ostertagia ostertagi*. Within 3–4 weeks of experimental infection (Canals and Gasbarre, 1990; Mansour et al., 1990), and 2 months of exposure to infected pastures (Gronvold et al., 1992; Gasbarre et al., 1993; Nansen et al., 1993) previously naive calves show significant rises in anti-*Ostertagia* antibodies in the peripheral circulation. These antibody responses have been detectable using a wide range of parasite derived antigens, and involve all major immunoglobulin isotypes. At the same time, very extensive changes are being seen in the local tissues of the abomasum. In the first 3–4 days after infection, there is a significant increase in the size of the regional lymph nodes draining the abomasum, and by 4–5 weeks after infection, the weight of these nodes reaches 20–30 times that of the same lymph nodes taken from uninfected age and size-matched calves (Gasbarre, 1986, 1994; Canals et al.,

1997). This increase in size is a result in an increase in number of both parasite-specific lymphocytes, and lymphocytes that do not recognize parasite antigen (Gasbarre, 1986). In addition, the percentages of B lymphocytes in the nodes is higher indicating a preferential expansion of these cells, and there is a corresponding decrease in the percentage of T cells (Gasbarre, 1994; Canals et al., 1997). Similar changes are seen in the mucosa of the abomasum. Within 4 days of infection the number of lymphocytes that can be recovered from the abomasal mucosa increases eightfold (Almeria et al., 1997). As seen in the draining lymph nodes there is an increased percentage of B lymphocytes and a concomitant decrease in the percentage of T cells in both naturally (Baker et al., 1993b) and experimentally (Almeria et al., 1997) infected animals. The only difference being that this shift occurs sooner than observed in the regional lymph nodes. This implies that soon after infection, *Ostertagia* antigens are presented to the host in the draining lymph nodes, and that within the first 3–4 days of infection these cells have left the nodes, entered the peripheral circulation, and have homed to tissues immediately surrounding the parasite where they become established.

Further examination of the responses elicited after infection reveals that the responses in many ways are similar to those seen in murine systems. There is a high level of expression of IL4 in both the draining lymph nodes and in lymphocytes isolated from the mucosa (Canals et al., 1997; Almeria et al., 1998). Unlike the murine model systems lymphocyte populations taken from *Ostertagia* infected cattle seem to be up-regulated for number of other cytokines, most notably Interferon-γ (IFN-γ), (Canals et al., 1997; Almeria et al., 1998). This is of note because IL4 and IFN-γ are considered to be counter-regulatory. This cross-regulation by IL4 and IFN-γ is considered to be one of the major factors driving immune responses to the Th1 and Th2 phenotypes. These results imply that in the case of *Ostertagia*, the immune responses elicited are not simply the stereotypic Th2 response seen in other GI nematode infections. In terms of the effector populations found in the tissues surrounding the parasites, *Ostertagia* again does not appear to be typical. Generally, nematode infections induce dramatic changes in the tissues surrounding the parasite. These changes include but are not limited to mucosal mast cell hyperplasia, generation of globular leucocytes, eosinophilia, increased mucus secretion and increases in the mass and activity of smooth muscle in the gut (Balic et al., 2000). Interestingly, naturally but not experimentally infected cattle were shown to have increased numbers of mast cells and eosinophils in the mucosal tissues (Baker et al., 1993a). A preliminary study in our laboratory has yielded similar results. In this study, calves given a secondary infection with *H. placei* were found to have increased numbers of mucosal mast cells and globular leucocytes. In contrast a similar challenge using *Ostertagia* did not increase the numbers of these cells in the tissues, but instead showed increased numbers of eosinophils (unpublished results).

The fact that *Ostertagia* infections appears to be very efficient stimulators of a number of lymphocyte subpopulations, but very poor inducers of effector cell populations indicate that *Ostertagia* has evolved means to suppress or evade protective immune mechanisms. A number of potential suppressive mechanisms have been proposed including: the generation of suppressor cells (Klesius et al., 1984), polyclonal activation the immune system (Gasbarre, 1986), and the elaboration of parasite products that regulate cell growth (De Marez et al., 1997).

4. Genetic control of resistance

Clearly the immunobiology of GI nematode infections in cattle is complex and the interactions between parasite and host immune system are multifaceted. It is plain that different parasites may induce different types of responses involving multiple effector mechanisms that are co-ordinated and tightly regulated. The parasites may also have evolved means to evade some or all of the responses elicited. The question then arises as to how are the host immune system can be manipulated to minimize losses caused by these parasites. Immunological tools such as vaccines or immuno-modulators may someday be useful in the protection of cattle from infection, at least with certain parasite species. At the present time the value of this approach is limited by the ability of nematodes such as *Ostertagia* to evade host immune responses. As these evasive mechanisms are better understood, procedures to ablate or lessen these immuno-regulatory activities can be devised. But a continuing problem with immunotherapy (i.e. vaccines or immuno-modulators) will be the need for the treatment to confer protection against all important parasite species in a given locality, and at the same time fit within producer guidelines regarding cost and labor required. Because of these current limitations on vaccination or immuno-modulation, the most attractive alternative or adjunct to anthelmintics is to identify and utilize disease resistance genes present in bovine germplasm to reduce parasite transmission.

Studies performed at our laboratory in conjunction with the University of Maryland's Wye Angus herd demonstrated that the number of nematode eggs/gram (EPG) in feces of pastured cattle was strongly influenced by host genetics (Leighton et al., 1989), and that the heritability of this trait was approximately 0.30 (Gasbarre et al., 1990). This value is similar to that reported for cattle by Stear et al. (1984) in Australia, and slightly higher than reported by Klooterman et al. (1992) in Europe, Zinssatag et al. (2000) in Africa, and Suarez et al. (1995) in South America. In addition, EPG values were not "normally" distributed and a small percentage of the herd was responsible for the majority of parasite transmission (Gasbarre et al., 1990). This "overdispersion"of EPG values was first described by Crofton (1971a,b) and has been reported in other cattle populations (Genchi et al., 1989). In this "overdispered" distribution, the value of the standard error of the mean exceeds the value of the mean, and as such most individuals have relatively low fecal EPG values, and a small percentage of animals, estimated to be between 15 and 25% of the total population (Anderson and May, 1985; Genchi et al., 1989), exhibit high EPG values. This pattern strongly suggests genetic management of a small percentage of the herd could considerably reduce overall parasite transmission. Results also indicated that the odds of certain bulls producing susceptible (high EPG) calves were 20 times greater than other bulls (Table 2) (Gasbarre et al., 1995).

To verify that these results were generally applicable to cattle raised in an area with different parasite fauna and transmission conditions, semen from high and low EPG bulls were used to inseminate cows at the USDA Subtropical Agricultural Research Station (STARS) in Brooksville, FL. Progeny tests confirmed the EPG phenotypes inferred from the bull estimated progeny difference (EPD) were generally similar under the two different management programs (Hammond et al., 1997). Based upon these findings a selective breeding program was initiated in our laboratory using parental stock originating from the Wye Angus herd. Although studies indicated that the bovine major histocompatibility

Table 2
Odds ratio for producing "High" EPG or serum pepsinogen calves from selected sires

Animal No.	No. of calves	Percent high EPG	EPG odds ratio	Pepsinogen odds ratio
1	17	0	0.27	0.19
2	9	0	0.51	0.21
3	23	13.0	0.80	1.17
4	19	15.8	1.00	1.00
5	13	38.5	3.20	0.98
6	65	41.5	3.74	0.79
7	8	50.0	4.94	2.99

complex (BoLA) had little or no effect upon EPG phenotype (unpublished), nonetheless animals were bred to be homozygous across the BoLA complex to minimize effects of this locus and more importantly provide flexibility for immunological assays. Serological reagents were produced that recognized all BoLA Class I and Class II determinate products within the Wye herd. A haplotype that was designated A11 Dx3 was chosen because: (1) this haplotype had been found in all *Bos taurus* breeds tested; (2) these Class I and Class II alleles were seldom found associated with other alleles; (3) the haplotype was common in the herd allowing selection of many natural homozygotes; (4) both high and low EPG types could be found bearing this haplotype. Once the initial breeding females were identified, semen from high and low EPG bulls was used to produce calves of the desired phenotypes. Calves were kept with their dams on pastures with extremely low numbers of parasites prior to weaning. When the median age of the contemporary group was 205 days, calves were weaned and placed on pastures infected with the two most common nematode parasites of US cattle, *O. ostertagi* and *C. oncophora*. The calves were monitored weekly for the following: fecal EPG, serum pepsinogen level, serum antibodies of the IgG1, IgG2, IgA and IgM subclasses to *Ostertagia* and *Cooperia* crude antigens, blood eosinophil levels, complete blood count (CBC) (hematocrit, hemoglobin, red blood cell count, white blood cell count, and mean cell volume), body weight, hip height, and scrotal circumference of the bull calves. The calves were kept on pasture for a minimum of 120 days, and animals were selected as replacement breeders, for re-challenge experts, or for immediate kill for collection of parasitologic and immunologic data. Data collected at kill included: parasite species and numbers recovered, sex and length of worms, enumeration of *Ostertagia*- and *Cooperia*-specific T cells in the abomasal and mesenteric lymph nodes by limiting dilution analysis, weight of abomasal lymph nodes, enumeration by flow cytometry of CD3, CD4, CD8, IL2-receptor, B-cell marker, surface IgM, and γ T cell receptor positive cells in the abomasal and mesenteric lymph nodes, and semi-quantitative competitive PCR measure of mRNA expression of IL2, IL4, IL10, IL13, IL15, IL18, $\gamma-$IFN, TNFα, and TGFβ in abomasal and mesenteric lymph nodes.

As these animals were characterized, correlations between phenotypic measurements, parasite load, and immune response became evident. The best indicator of the number of *Cooperia* infecting the host was the EPG value ($r = 0.6$), while *Ostertagia* was best measured by serum pepsinogen levels ($r = 0.7$), weight gain ($r = -0.5$), and measures of anemia ($r = 0.5$). Other phenotypic measures were either not significantly associated

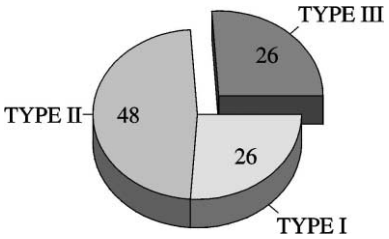

Fig. 1. Percentage of calves exhibiting different EPG excretion profiles.

with parasite numbers or were very weakly correlated (Gasbarre, 1997). These relationships are in general agreement with those reported in similar parasite–host systems (Eysker and Ploeger, 2000; Claerebout and Vercruysse, 2000). In contrast, studies in tropical areas have indicated a significant correlation between fecal EPG values and total worm numbers (Bryan and Kerr, 1989).

In addition, calves could be separated into three types: (1) Type I never demonstrated high EPG values, (2) Type II showed rises in EPG values through the first 2 months of the test then the EPG fell and remained at level associated with the Type I calves; (3) Type III calves maintained high EPG levels throughout the test. The approximate percentage of these calves through the first generation of breeding was 25:50:25, respectively (Fig. 1). A secondary infection experiment revealed that calves of the Type I and Type II EPG phenotypes maintained low EPG values, while calves of the Type III EPG phenotype continued to shed high number of eggs. These EPG phenotypes can be categorized into three distinct classes: innately immune; acquired immune; immunologically non-responsive. From a practical standpoint, control of parasite transmission and parasite-induced loss, should be based on targeting these non-responder class of animals.

To date, 288 calves have been phenotyped by these methods and fourth generation calves are being produced. Recent analysis of these data indicate selection for calves in the innately immune and the immunologically responsive groups has occurred. In addition, the herd EPD range is equal to ∼50% of the grand mean EPG value for all calves tested to date (unpublished data). These results demonstrate that: (1) host genetics account for a substantial amount of the variation in EPG values; (2) very significant change in parasite transmission patterns can be affected by manipulation of the host genome.

5. Identification of genes controlling resistance

Genomic science holds great promise for providing research tools that identify and elucidate host genetic components of GI nematode infection in cattle. Some of this research will benefit cattlemen, if the results can be incorporated into current genetic selection programs in a cost effective manner. The Angus cattle described in the previous section are segregating for traits involved in resistance and susceptibility to GI nematodes, and as such, this resource population can be used to effectively detect the genomic locations of these economic trait loci (ETL). Several components are required for a genome-wide analysis

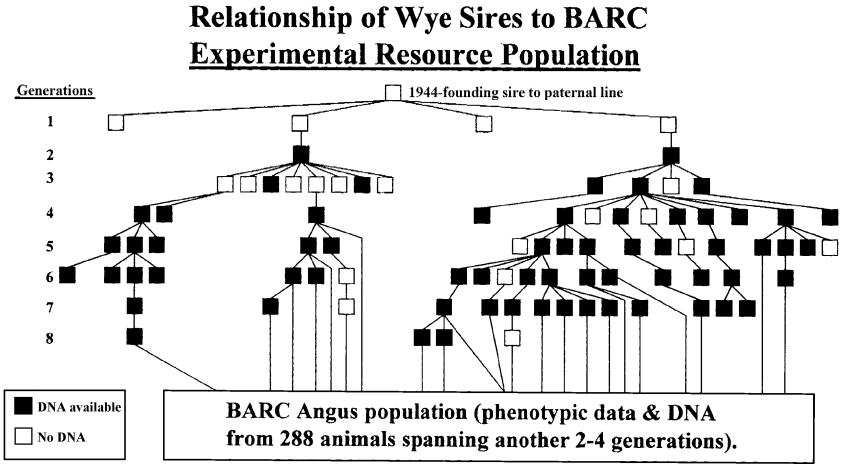

Fig. 2.

approach to identify and reliably map ETL. These factors include: complete and accurate records of heritable phenotypes; full pedigree information; marker genotypes from regular intervals throughout the entire genome and proper analysis algorithms.

In the case of the BARC Angus population, complete phenotypic data for over 50 traits are available for the nearly 300 progeny generated over the past 9 years. Heritability estimates were moderate based on the direct measurement of nematode eggs (Gasbarre et al., 1990). Heritabilities for other traits will be calculated using MTDFREML (Boldman et al., 1995). The complete pedigree records for this population as well as for ancestors in the Wye herd are available. Initial pedigree analysis of the resource population revealed that >90% of the animals were paternally descended from a Wye bull born in 1944 (Fig. 2). Genomic DNA was collected from all the parents and progeny (over 400 animals), and these samples were used to generate marker genotypes from 197 DNA markers spaced at regular intervals (~20 cM) across the entire genome (3000 cM). Markers were selected from the USDA Meat Animal Research Center reference linkage map (http://sol.marc.usda.gov/genome/cattle/cattle.html). This reference map is composed mainly of microsatellite (MS) markers, and MS are not typically associated with genes. MS markers have a higher polymorphic information content than markers associated with single nucleotide polymorphisms, and as such, these markers remain the best way to track inheritance in the complex pedigrees of the BARC Angus resource population. Preliminary analysis of average MS marker heterozygosity index ($N = 23$ markers) for the resource herd parents ($N = 40$ animals) was >50%, indicating that enough MS markers were available to produce a genome wide analysis of this population (Stone and Keele, unpublished). Markers associated (<1 cM) with 12 candidate cytokine genes involved in growth and differentiation of bovine T-cells have been added to the scan to enhance rapid detection of ETL (Sonstegard, unpublished). The decision to analyze these genes prior to completion of the genome scan is based on the strong association between IFN-γ haplotypes and resistance to a species of GI nematode in sheep (Crawford, 1998).

For relational analysis between phenotype and genome location, over 80,000 genotypes have been generated by PCR amplification, and marker genotypes have been scored to produce inheritance data. The marker allele inheritance data is currently being statistically analyzed to detect patterns of co-segregation between allele haplotype and EPG phenotypes. Statistical power of this genome-wide scan has been strengthened by including genotypic data from the historic pedigree. Complex pedigrees, especially those containing loops, have posed a challenge for data analysis to detect ETL. Research on these problems at MARC has resulted in algorithms and software implementing a multiple locus allelic peeling algorithm (Thallman et al., 1999a,b). Using these tools will allow reconstruction of inheritance from haplotypes of founding sires even when DNA is not available for those animals. This complex pedigree analysis increases statistical power compared to within family analyses, especially when family size is small. In our herd, paternal half-sib families range from 5 to 13 progeny/sire. Extensive marker genotypes are available from ancestors (over 70 historical sires) of the population most of which were paternally descended from a single founding sire of the Wye herd (Fig. 2). This characteristic of the BARC herd unintentionally resulted from previous selection for a single MHC haplotype.

Once ETL have been identified, the next step will be to refine ETL map resolution in an attempt to discover the genes underlying disease phenotypes. Accurate identification of genes controlling resistance will offer the producer several alternatives for disease control. For a non-organic producer, the small percentage of susceptible animals can be targeted for drug administration. This approach would reduce both the cost of anthelmintics used and the odds for selection of drug resistant mutants, because the selective agent (drug) would not be applied over the entire parasite population. A second treatment option would be based on correcting a heritable immunologic condition. In this case, susceptible animals could be the targets for immunotherapy involving vaccines or immuno-modulation. A final option would be genetic selection to remove susceptible animals from the herd. Producers with a high degree of risk for parasite-induced production losses, such as organic producers or producers in geographic areas with environmental conditions favorable to high rates of transmission would benefit the most from this strategy. In contrast, producers at low risk could take a more conservative approach and select against susceptibility when other factors were equal.

6. Summary

GI nematode parasites of cattle remain a serious impediment to the efficient production of milk and meat throughout the world. Although there are several classes of very effective and safe anthelmintic drugs, a number of factors including the appearance of anthelmintic resistant parasites, the growing organic movement, and the cost of drugs in less affluent areas of the world, make it important that alternate or adjunct control measures be available. One of the most attractive is to use the host immune system to control the impact of disease. Unfortunately, immune responses elicited by nematode infections are very complex and highly regulated. Almost all GI nematodes elicit a very stereotypic immune response characterized as a Th2-like response, i.e. one involving the elaboration and secretion of IL4. While these responses are effective in protecting from infection of

some nematode species, it is clear that the most economically important species are those that have found ways to circumvent this typical immune response. Because of our lack of understanding of the complexity of the protective immune responses it will be difficult to devise immuno-therapeutic programs based on vaccines or immuno-modulators. An alternative is to use the natural diversity of the host genome to develop management programs to minimize parasite transmission. Within a relatively short time period, most pastured animals develop immune responses that limit parasite egg output. A few animals remain susceptible and serve as the focus for parasite expansion in the herd. A significant factor in this state of "non- responsiveness" is the genetic make-up of the host. This diversity of genetically controlled responsiveness makes it possible for us to begin to identify the host genes which control immunity to the parasites. A successful search for resistance/susceptibility genes is dependent upon having a number of resources available. These resources include an animal population with a carefully defined pedigree, an accurate measure of the trait of importance, and tools and reagents to map the trait within the bovine genome. Once genes or markers for the genes that determine resistance are identified, the opportunity exists to determine the exact mechanisms of immunity, to define how immunity is regulated, and to develop practical control programs based on parasite resistance.

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