International Journal for Parasitology 42 (2012) 647–655

Contents lists available at SciVerse ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Immuno-epidemiology of chronic bacterial and helminth co-infections: Observations from the field and evidence from the laboratory

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ARTICLE INFO

Article history:
Received 1 February 2012
Received in revised form 21 March 2012
Accepted 12 April 2012
Available online 11 May 2012

Keywords:
Bordetella bronchiseptica
Graphidium strigosum
Co-infection
Immune response
Laboratory infection
Field epidemiology

ABSTRACT

Co-infections can alter the host immune responses and modify the intensity and dynamics of concurrent parasitic species. The extent of this effect depends on the properties of the system and the mechanisms of host–parasite and parasite–parasite interactions. We examined the immuno-epidemiology of a chronic co-infection to reveal the immune mediated relationships between two parasites colonising independent organs, and the within-host molecular processes influencing the dynamics of infection at the host population level. The respiratory bacterium, *Bordetella bronchiseptica*, and the gastrointestinal helminth, *Graphidium strigosum*, were studied in the European rabbit (*Oryctolagus cuniculus*), using long-term field data and a laboratory experiment. We found that 65% of the rabbit population was co-infected with the two parasites; prevalence and intensity of co-infection increased with rabbit age and exhibited a strong seasonal pattern with the lowest values recorded during host breeding (from April to July) and the highest in the winter months. Laboratory infections showed no significant immune-mediated effects of the helminth on bacterial intensity in the lower respiratory tract but a higher abundance was observed in the nasal cavity during the chronic phase of the infection, compared with single bacterial infections. In contrast, *B. bronchiseptica* enhanced helminth intensity and this was consistent throughout the 4-month trial. These patterns were associated with changes in the immune profiles between singly and co-infected individuals for both parasites. This study confirmed the general observation that co-infections alter the host immune responses but also highlighted the often ignored role of bacterial infection in helminth dynamics. Additionally, we showed that *G. strigosum* had contrasting effects on *B. bronchiseptica* colonising different parts of the respiratory tract. At the host population level our findings suggest that *B. bronchiseptica* facilitates *G. strigosum* infection, and re-infection with *G. strigosum* assists in maintaining bacterial infection in the upper respiratory tract and thus long-term persistence.

1. Introduction

Natural animal populations are frequently colonised by a community of parasitic species that may exhibit persistent, life-long infections. Parasites that elicit chronic infections have developed strategies to inhibit, divert or adapt to host constraints and in so doing procrastinate or avoid expulsion and optimise fitness (Monack et al., 2004; Blaser and Kirschner, 2007; Allen and Maizels, 2011; Babayan et al., 2010; Bourke et al., 2011). For hosts infected with parasite species that colonise independent organs, where there is no direct parasite competition, the main route of interaction is via the host immune system (Cox, 2001; Page et al., 2006). Co-infection with a second parasite species can increase, decrease or have no effect on the establishment and long-term survival of the first parasite inhabiting a different organ, either because the first parasite is able to overcome or adapt to the immunological changes caused by the second parasite species or because these changes are too weak to influence the dynamics of the first infection (Cox, 2001; Graham, 2008; Thakar et al., 2012).

Bacterial–helminth co-infections are often associated with greater bacterial proliferation and increased tissue damage, caused by the impairment of the protective type 1 (Th1) immune response against the bacteria by the development of type 2 (Th2) responses to the helminths (Brady et al., 1999; O'Neill et al., 2001; Supali et al., 2010; Perry et al., 2011). For instance, chronic infections with *Onchocerca volvulus* suppressed the cellular responses to *Mycobacterium tuberculosis* and *Mycobacterium leprae* antigens and promoted active bacterial infections in humans (Stewart et al., 1999). Similarly, *Heligmosomoides polygyrus* increased *Helicobacter pylori* colonisation and chronic inflammation in mice through down-regulation of the protective Th1 immune response to the bacterium (Fox et al., 2000). From a different perspective, our recent work on chronic co-infections with the respiratory bacterium *Bordetella*

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0020-7519/$36.00
http://dx.doi.org/10.1016/j.ijpara.2012.04.011

bronchiseptica and the gastrointestinal helminth *Trichostrongylus retortaeformis* in rabbits showed that *B. bronchiseptica* accelerated the expulsion of *T. retortaeformis* from the small intestine while the helminth had no effect on bacterial clearance from the lung but enhanced bacterial numbers in the nasal cavity (Thakar et al., 2012). This study supported the hypothesis of helminth-mediated down-regulation of protective immunity against bacterial infections but also emphasised the contrasting responses of organs infected with the same parasite and the role of bacteria in promoting helminth expulsion. The modulatory role of bacteria in helminth intensity is not without precedent. *Mycobacterium bovis* has been shown to increase *Strongyloides venezuelensis* intensity in mice and this was associated with a decreased immune response to the helminth, specifically low IL-4 and IL-13 cytokines, and an enhanced IFN-γ expression to the bacterium (Carmo et al., 2009).

By altering the susceptibility of the host to a second infection, concurrent infections can play a critical role in modulating parasite transmission and disease prevalence at the host population level. For example, the prevalence of chronic leprosy in humans was found to double in microfilaria hyperendemic regions (Stewart et al., 1999). In the case of sexually transmitted diseases, HIV shedding increased in humans co-infected with *Neisseria gonorrhoeae* and potentially amplified the risk of disease spread (Rotchford et al., 2000). Variability among hosts in age, sex or breeding status can influence the immune responses and contribute to seasonal variation in prevalence and intensity of co-infections and ultimately long-term disease dynamics (Cattadori et al., 2007, 2008; Tornatore et al., 2012). Nevertheless, how co-infections alter the intensity and dynamics of single infections at the population level has still to be clearly addressed in the case of persistent infections. While immunological studies have been very successful in disentangling some of the key molecular mechanisms during multiple parasite species infections, the epidemiology of co-infection is still very much undeveloped and restricted to theoretical studies or to a few human diseases. A better understanding of the immuno-epidemiology of chronic co-infections requires an appreciation of the mechanisms of host–parasite and parasite–parasite interactions during the acute as well as persistent phase of the infection, and how these within-host molecular processes can explain the dynamics of both parasites in the host population (Hellriegel, 2001; Dunne and Riley, 2004).

We used a combination of field monitoring and laboratory experiments to examine the concurrent infections of the respiratory bacterium, *B. bronchiseptica*, and the stomach helminth, *Graphidium strigosum*, in the European rabbit (*Oryctolagus cuniculus*). The general aim of this work was twofold. First, the dynamics of single and co-infections in a free-living rabbit population was explored to identify seasonal trends and changes in prevalence and intensity of co-infection with host age. Second, the immune responses against both parasites were quantified in the laboratory and these findings were used to explain the patterns of infection observed in the natural setting. We predicted that *G. strigosum* would down-regulate the immune response against *B. bronchiseptica* and so enhance bacterial numbers in the oral–nasal cavity and delay clearance in the lower respiratory tract, relative to single bacterial infections. In contrast, we predicted that *B. bronchiseptica* would alter the immune response to, and abundance of, *G. strigosum* but would not eliminate the helminths from the stomach, as there is no evidence of immune-mediated clearance for this helminth (Murphy et al., 2011). In the field, we expected *B. bronchiseptica–G. strigosum* infections to be common and to increase with rabbit age. Specifically, we predicted that the occurrence of co-infected hosts would increase the prevalence of both parasites, although showing different seasonal patterns of infection. Findings from this study provide fundamental knowledge that has relevance for the rabbit as well as for agricultural animals and wildlife that share the same respiratory bacterium and a closely related community of gastrointestinal helminths, namely cattle and sheep.

2. Materials and methods

2.1. The host–parasite system

Bordetella bronchiseptica is a common bacterial infection of the respiratory tract of a large number of wild and agricultural animals (Diavatopoulos et al., 2005). Infections are persistent with severity ranging from asymptomatic to pathogenic and occasionally fatal in co-infections with other bacteria or viruses (Baker, 1998; Brockmeier, 2004; Brockmeier et al., 2008; Rougier et al., 2006). In agreement with previous studies on mice and rats, we showed that singly-infected and helminth co-infected rabbits removed *B. bronchiseptica* from the lower respiratory tract but were unable to clear the infection from the nasal cavity where bacteria persisted in high numbers throughout the challenge (Pathak et al., 2010; Thakar et al., 2012). The modelling of the immune responses suggested that both in bacterial and bacteria–helminth infections, expulsion from the lungs was mainly driven by a type 1 response, in which IFN-γ mediated antibody production and neutrophil recruitment led to bacterial phagocytosis (Thakar et al., 2012).

Graphidium strigosum is a nematode with free-living stages and a direct life cycle, colonising the stomach of the European rabbit (*O. cuniculus*) and to a lesser extent other lagomorphs (Newey et al., 2005; Eira et al., 2007; Massoni et al., 2011). In temperate regions, infections are highly seasonal and affected by the emergence of naïve offspring during the spring–summer months (Boag et al., 2001; Cattadori et al., 2008). Intensities accumulate exponentially with host age, a pattern consistent among cohorts of hosts born in different months (Cattadori et al., 2008). To confirm these field observations, a primary laboratory infection showed that *G. strigosum* persisted with high intensities throughout the experiment, despite a strong mucosal IL-4 response and increasing serum antibody levels (IgA and IgG), although mucosal antibodies were relatively low (Murphy et al., 2011).

2.2. Field monitoring

Data on *B. bronchiseptica* and *G. strigosum* infection were available from a free-living population of European rabbits sampled monthly from 2004 to 2010 in Perthshire (Scotland, UK). Rabbits were collected for pest control purposes following UK regulations and all procedures were approved by the Institutional Animal Care and Use Committee of The Pennsylvania State University, USA. Details of rabbit sampling, parasite quantification and antibody measurement are described elsewhere (Cattadori et al., 2005, 2008; Pathak et al., 2011a; Cattadori et al., unpublished data). Briefly, an individual was considered *B. bronchiseptica*-positive when the antibody O.D. was above the average O.D. of sera from uninfected laboratory rabbits (Pathak et al., 2011a). Based on our previous work, we made the assumption that rabbits seropositive to *B. bronchiseptica* carried an active infection (Pathak et al., 2010; Thakar et al., 2012). IgA and IgG against *G. strigosum* were also quantified and based on excretory/secretory adult helminth products (ES) as a source of antigen (Cattadori et al., unpublished data). Helminth ES by-products are commonly used as an alternative to somatic homogenates (Hewitson et al., 2009); we chose the first over the second as they exhibited negligible cross-reactivity with *T. retortaeformis*, the most prevalent helminth in our rabbit population (Cattadori et al., unpublished data). A rabbit was *B. bronchiseptica–G. strigosum* co-infected if showing an active helminth infection and bacterial sero-conversion above the control cut-off O.D. threshold.