Journal of Helminthology (2003) **77,** 147–153 DOI: 10.1079/JOH2003176

Causality or coincidence: may the slow disappearance of helminths be responsible for the imbalances in immune control mechanisms?

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Abstract

Intestinal infection continues to be a problem worldwide and helminths, which currently infect billions of individuals, are primary culprits. The major burden of disease falls on the populations of developing countries, given that over the last four to five decades helminth infections are disappearing in industrialized societies. In developing countries, a major source of immunomodulatory signals in post-natal life are parasites, particularly helminths, which, unlike most bacteria and viruses, selectively stimulate Th2 function. Helminths and their eggs are probably the most potent stimulators of mucosal Th2 responses. Responses elicited by worms can modulate immune reactions to other parasites, bacterial, viral infections and several unrelated diseases. Bacterial and protozoal infections may also protect against atopy and asthma, through the induction of the Th1 regulatory responses. Today, people in developed countries often live in ultra-hygienic environments, avoiding exposure to viruses, bacteria, ectoparasites and endoparasites, particularly helminths. Perhaps failure to acquire worms and experience mucosal Th2 conditioning predisposes to unrelated diseases. In contrast to this hypothesis it has also been suggested that Th2 responses can make the host more susceptible to other important diseases and to contribute to the spread of them.

Introduction

To determine whether the gradual disappearance of helminths should be seen as an epiphenomenon or not, it may be useful to briefly examine past and present human/environment interactions and the consequent development of disease. For the purposes of this review, the environment will be considered in broad terms as the boundary delimiting an 'adaptation' process, i.e. the interaction/integration that has developed over time between organisms and the world in which they live. The environment is therefore the product of a dynamic complementarity which results in the establishment of new, though not always optimum, equilibria between organisms and environment. One example of a less than optimum outcome of this adaptation is where the environment now acts as a trigger of disease.

Parasites, especially helminths, are intriguing examples of this sometimes conflicting rather than compatible interrelationship. It is no coincidence that the term parasitism is defined as a peculiar kind of association between organisms of different species. It is used to describe those cases where one species lives in continuous intimate association with another species, on which it depends to some extent for its metabolic requirements. It is widely accepted that the parasite alone benefits from this kind of partnership, but over the last ten years or so, that is since the advent of the 'hygiene hypothesis' (Strachan, 1989) many questions have been raised in an attempt to understand whether the 'host' also derives or has derived some advantages (Bundy *et al.*, 2000).

For millions of years parasite evolution has been directed towards complementarity with a host in order to

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survive and reproduce. Through a variety of forms of specific contact, parasites have developed successful strategies for the survival of the species. This involves the selective choice of an environment and the identification of a complex relationship of interactions both without and within the host. For each parasite and its selected host there is a complex response system characterized by the search for a mutual equilibrium that ensures survival and multiplication of both parasite and host. This is much more than the sum of the individual interactions but represents a network of sophisticated interactions (Wakelin, 1984; Maizels *et al.*, 1993). In this complex system the two actors behave complementarily, not as organisms in conflict but as organisms coexisting in a network of relationships. In this context the environment can be seen as an ensemble of organisms interacting in cooperation, symbiosis, and parasitism, of selection, competition and adjustment. In nature this situation appears as a rational series of interconnections, that has produced over evolutionary time a wide variety of adaptations, constrained only by the basic limitations of biological organization. Within this scenario, which is one of dynamic equilibrium, the appearance of a new disease or the unexpected spread of known diseases in a given population represents an anomaly, a departure from a trend that attracts the attention of researchers.

Key questions

There are two key questions to be answered:

1. If the organism has evolved over time towards a balanced relationship with its environment, using this in the broad sense, what can possibly have altered this relationship?
2. What factors can upset, in the space of a few decades, an equilibrium that required millennia of adaptation to attain?

A possible answer has been provided by the environmental or hygiene hypothesis. Essentially this considers that the slow but continuous decline of infection by intestinal helminths and by many ectoparasites, and the reduction of morbidity and mortality of infectious diseases, brought about by changing life styles, have resulted in disorders in immune control and consequently an increase in non-related diseases.

Populations of species have always been confronted with adverse situations to which they have adapted over long periods of evolutionary time. For humans, over the last 50 years, and especially in the western world, there has been a departure from such adaptive trends for which no evolutionary interpretation can be found, because it has happened in too short a time scale. This situation can be interpreted as an imbalance (a search for a new equilibrium in too short a time) that has given rise to a new equilibrium with deleterious effects which have led to the emergence of new or the re-emergence of old diseases.

Biological and social causes

At this juncture it may be interesting, in the light of the large number of published papers, to examine the potential biological and social causes of this situation, and in the context of the epidemiological data, attempt to find an *a priori* causal explanation (although this is subjective and hypothetical).

The coincidences described in the literature are intriguing. However, although in the present authors' view the slow disappearance of the helminths and the emergence of non-related diseases appears more than a coincidence, it is difficult to demonstrate a direct causal link. Advances in medicine and in helminth control have undeniably contributed to suppressing diseases that keep others in check and hence to altering the ecological balances created over millennia. Sociological changes, by altering exposure to environmental antigens, have also contributed at the individual level to interfering with antigen recognition mechanisms and consequently to disrupting immune response mechanisms.

Such changes were not readily recognized, because, in the laboratory, host/parasite models were studied with a view to boosting immune response mechanisms and not to assessing their presumed utility for the host. It is true that the ability of the parasite to evade the host's response has always fascinated parasitologists, one good example being the tapeworm *Hymenolepis diminuta* in the rat, which is known to survive as long as its host (Hopkins, 1980; Palmas *et al.*, 1993) and, most surprisingly, improves the host's trophic state compared with non-infected controls (C. Palmas, personal observation).

Initially, the hygiene hypothesis was based on data from paediatric clinics which were the first to notice the unexpected occurrence of certain atopic diseases, and the sudden rise in asthma cases in spite of early diagnosis and improved treatment. The increased incidence of these diseases exhibits a peculiar trend, both in epidemiological and clinical terms (Shirakawa *et al.*, 1997). Clinical observations suggested a possible immune malfunction of cells associated with frequent and widespread antigen exposure in genetically predisposed individuals (Gale, 2002). These data provided the first enigmatic and unusual evidence.

No totally satisfactory explanation appears to have been proffered for this situation. However, the hypothesis of a co-factor or 'promoter' that, when present above a certain critical threshold, could control the spread of other diseases and, which, when absent, became an actual cause of disease, has gained momentum. In one hypothetical biological model the absence of parasites, or at least parasites like helminths, appears to be the missing factor involved in the spread of new diseases. Presumably the combined effect of the disappearance of intestinal helminths and sociological changes creates an immune imbalance that, in genetically predisposed subjects, facilitates the appearance of non-related diseases.

We can accept as plausible a multi-factorial hypothesis that brings into play the environment, changes in alimentary habits, 'disappearance' of the parasites, introduction of multiple vaccinations and new synthetic molecules (drugs, pesticides, etc.), pollution and genetic characteristics. Scepticism about this explanation concerns the environment in the broad sense. The first epidemiological data related to industrialized countries, where the most significant and traumatic changes occur. But how are we to know whether in a genetically

predisposed subject the 'changed' environment is responsible for an immune imbalance?

The multiplicity of factors making up the environment, and the 'complexity' of the adaptation process that has developed over time, has driven the search for specific promoters or co-factors that are able to alter the immune response in genetically predisposed subjects. On closer consideration, it would appear that the multifactorial hypothesis can be supported by examining the different components concerned; viruses, bacteria and parasites, the competition and complementarity among them, between them and the environment, between them, the environment and the genetic heterogeneity of different populations. This hypothesis is borne out in part by the clinical expression of type I diabetes in Sardinia and north European countries where incidence rates are practically identical (about $36\%_{000}$ inhabitants) (Muntoni & Songini, 1992). The disease affects homogeneous population groups in terms of genetic susceptibility. Yet, the disease-predisposing genetic background differs in these two macro-populations (Lernmark, 2002).

The geographical distribution of diseases that are increasing should be related to the different changes introduced by man and in particular, childhood diseases should receive priority attention. Those diseases that are inexplicably on the increase today may arise through none other than the expression of normally latent conditions, awaiting activation by a 'promoter' such as introduced substances (e.g. drugs or vaccines). Alternatively they may be generated by the absence of a 'repressor', i.e. the disappearance of viruses, bacteria or parasites having immunomodulatory activities. Based on epidemiological studies, researchers in the various disciplines involved (parasitologists, immunologists, virologists, etc.) have begun to explore the possible underlying causes of infections and atopy. Attention is being focused on isolated diseases or on groups of similar diseases, reducing this complex problem into discrete factors, in order to simplify study and analysis. However, to use Grmek's words, this is creating an epistemological obstacle: 'This kind of situation is well known in the history of science, an epistemological obstacle looms before researchers, created by their own studies, by the prejudices that necessarily accumulate when they pursue a given research direction' (Grmek, 1989). Researchers, by following the fashion or pursuing a reductive and/or mistaken hypothesis, are rejecting a multidisciplinary approach to basic and applied biomedical research, the only means of providing illumination and inspiration.

We can maintain that the coexistence of helminths in the human intestine may have proved useful, but their current 'absence' may not be the sole and sufficient cause of the rise of other diseases, as many other important factors have also influenced the history of mankind's health over the centuries. 'The absence of helminths', an event seemingly of little significance, has come, with the quantitative changes of other diseases, to represent a discontinuity, a departure from a millenary equilibrium that necessitates a search for new stability: a new re-equilibrium between biological and ecological factors.

At this point it would be opportune to focus attention on one factor: the role played by what Grmek defines as pathocoenosis, in other words the equilibrium in the frequency of all those diseases that affect a given population. Based on this assumption, the frequency of every disease depends, not only on the host organism, but also on the environment and on the frequency of other diseases in the same population (Grmek, 1969a,b). Human beings living in the different regions of our planet share all the diseases of that area, the ensemble of viruses, bacteria and parasites. The co-existence of all these microorganisms has produced, with the passing of time, those balances that are the result of the dynamic evolutionary complementarity between organisms and their environment, and that translate into the exchange of 'information' on antigenicity and on the potential to adapt to the various niches.

Data about the dynamic history of each pathocoenosis can easily be overlain by current quantitative changes (Grmek, 1969b, 1985). In general, the pathocoenosis is observed to change after each traumatic event (man-made or otherwise) resulting in the emergence of new diseases (qualitative change) or increasing the spread of known diseases (quantitative change). These diseases seem to concentrate selectively in genetically predisposed subjects.

Throughout the history of mankind, the balance of pathocoenosis in the western world has been disrupted at least four times:

1. In the Neolithic age – the transition to sedentary life.
2. In the Late Middle Ages – migration of Asiatic peoples.
3. Renaissance – discovery of America.
4. The present – a spatial-temporal, multifactorial fusion of exposure to pathogenic organisms, vaccinations and drug treatment decline in viral, infectious and parasitic diseases, disappearance of some diseases, and changes in alimentary habits.

Over the last two centuries, as a consequence of high childhood mortality from a variety of causes, the surviving adult population has borne the brunt of the effects brought about by changes in the patterns of prevalent diseases. The increase in immune and genetic diseases over the last ten years is an exception to this rule, because they appear more evident among children that currently have a greater likelihood of surviving the critical early years of life.

The success of efforts over the last century to combat infectious diseases has almost doubled average life expectancy in the western world. In the 1950s–60s improved standards of living, chemotherapy and vaccination with the Calmette-Guerin vaccine have accelerated the declining incidence and disappearance of tuberculosis, so that by the 1970s primary tuberculosis had become relatively rare. More interestingly, from our point of view, is the eradication of poliomyelitis as the result of mass vaccination. The generally improved standards of living in the western world also saw the beginning of the decline of intestinal helminths, though like many other pathogens, they persist in clinically asymptomatic forms.

Throughout its evolution and up to the mid 1900s the human species was steeped in a broth of antigens, allergens, enteropathogens and ectopathogens. This continuous, exposure moulded the biological

https://doi.org/10.1079/JOH2003176 Published online by Cambridge University Press

characteristics of the human beings we are today. Major improvements in hygiene and health standards, the use of drugs, vaccinations have disrupted the equilibrium of the pathocoenosis. And the parasites that for centuries coexisted with humans are now disappearing or retreating to small niches.

Developing countries

The same cannot be said of the developing countries where parasite-related diseases remain common and can still prove fatal (Bundy *et al.*, 2000). Helminth infections in male children begin to appear as soon as they start to crawl, while female children become infected later and their level of infection is not as high as their male peers (The Partnership for Child Development, 1997; Bundy *et al.*, 2000). From this time on and for many years, in some cases for their whole life, these children will be repeatedly reinfected (Bundy *et al.*, 2000). The parasite burden appears, at least in our experience, to be balanced and only in rare cases are massive infections (often caused by other diseases) observed.

In laboratory experiments mimicking similar natural infections we have demonstrated that the immune response intensifies as parasite load increases, but when this burden becomes excessive then the response can be disrupted and often ineffective (Ferretti *et al.*, 1980; Gabriele *et al.*, 1986; Bortoletti *et al.*, 1992). Therefore, high-level infections with their anomalous results were defined some 30 years ago by G. Ferretti as 'pathology of the pathology'. For this reason, parasites have developed sophisticated self-regulating mechanisms to evade the immune response and to avoid being destroyed by the host.

In this chronic and intimate association continual exposure to helminth excretory/secretory antigens modulates the host's immune response, shifting the balance in the direction of the powerful Th2 arm, associated with eosinophilia, secretion of IgE and, in some cases, mastocytosis. It is not clear whether this response is actually detrimental to the parasite in humans and today we are rather more interested to find out whether this response produces an impact on the host's immune system that alters its susceptibility to non-correlated diseases.

One would have expected a boost in the allergic response elicited by helminths, but the epidemiological data show that because of the high levels of non-specific IgE and down regulatory Th2 cytokines an adaptive protection against inflammatory disease is developed. These findings may explain the low incidence of atopic diseases in countries where helminths are still prevalent (Gale, 2002). For example, children infected with parasites have a mild skin reaction to *Ascaris*, but this improves after efficacious anti-helminth treatment, and the same down-regulated trend is observed for other common allergens (Lynch *et al.*, 1993).

In children infected with *Schistosoma*, high IgE directed against dust mites corresponded with weak skin sensitivity reactions, an effect mediated by high levels of the anti-inflammatory cytokine IL-10 (Van den Biggelaar *et al.*, 2000). Once the helminths had been eliminated, a marked increase in skin reactivity to common allergens (Lynch *et al.*, 1993) and of asthma cases (Yazdanbakhsh *et al.*, 2001) was observed in all patients.

It should be remembered that bacteria and protozoa can also protect against allergies and asthma, but in this case the explanation may lie in their involvement in inducing Th1 responses (Lynch *et al.*, 1999). The suppression of the Th1-mediated immunopathology has been demonstrated in clinical studies on humans (unnatural host) treated with *Trichuris suis* to alleviate the symptoms of inflammatory bowel disease (Shirakawa *et al.*, 1997). The powerful immunomodulatory effect of parasite infections by down-regulating certain allergic and inflammatory responses, would explain why a consequence of the slow decline of helminth infections has been the rise of atopic diseases in the developed world.

The example of Sardinia, an island in the Central Mediterranean provides some useful considerations in this regard. The Sardinian people are characterized by a peculiar and unique ethnic homogeneity, the result of centuries of partial isolation. In fact, genetic and anthropologic studies have demonstrated that despite being of Caucasian origin like the rest of the Italian and European populations, the Sardinians are a far more phylogenetically ancient branch (Songini, 1997). The uniqueness of their genetic make up makes the Sardinians a precious epidemiological resource for studying the dynamics of the events leading up to the development of a given disease. Up until the 1950s, Sardinia, with its subsistence economy (Attanasio *et al.*, 1985), presented a picture comparable with today's developing countries, an analysis of what happened in this macro-model or regional laboratory may therefore provide some very useful information.

Enterobius vermicularis

For most of their life, Sardinians of different social strata were commonly infected with intestinal helminths. The most well known and widespread was *Enterobius vermicularis*, and it is no coincidence that cures for this parasite are to be found also in popular medicine (Atzei *et al.*, 1994). Prevalence, estimated at between 40 and 60% of school children in the 1950s, was probably underestimated. Scotch tape tests carried out on a sample of 200 children staying at a seaside holiday camp in 1976 showed over 50% to be positive. Even in the 1980s in some schools in Cagliari, the island's largest city, more than 40% of children were found to have pinworms (Bortoletti, 1980; Bortoletti & Limongelli, 1985). Prevalence diminished in children aged between 11 and 13 from middle schools in some towns with less than 5000 inhabitants (Bortoletti & Satta, 1989). Social status and gender made no difference to infection levels and it was very common for some teachers to become infected; re-infection was fairly frequent, at least up to the 1960s, but the only data available are those gathered by family doctors. Our current data do not differ substantially from those for other European countries (Vermund & MacLeod, 1988; Kyrönsäppa, 1993; Herrström *et al.*, 1997; Gauert, 1998; von Ehrenstein *et al.*, 2000) which show a slow decline in pinworm infection. The data for *Enterobius* should be

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interpreted with some caution, because: (i) the parasite is ubiquitous; (ii) its decline is very slow in the western world; and (iii) it is the last ancestral colonizer of our intestine. However, infection levels are becoming increasingly lower and rates of relapse are also low.

The slow decline of this parasite has coincided with a rise in atopic diseases in all western countries. Data for immigrants appear to substantiate this hypothesis (Waites *et al.*, 1980; Von Mutius *et al.*, 1994, 1998; Cookson, 1999), and those for urban migration in some African countries likewise (Yemaneberhen *et al.*, 1997; Scrivener *et al.*, 2001). Can the disappearance of certain helminths, the virtually total decline of relapse and the increasingly lower numbers of pinworms present in the intestine trigger an immunological disorder?

In Sardinia the almost complete disappearance of parasitic diseases, and the eradication of polio, malaria and tuberculosis, have coincided with an increase in atopic diseases, in cases of asthma, coeliac disease, Crohn's disease, type I diabetes, and multiple sclerosis. Does the hygiene hypothesis hold within Grmek's concept of pathocenosis for all these diseases?

Can the intriguing NOD mouse model (Bowman *et al.*, 1994) provide valid experimental data to support this hypothesis? This mouse develops diabetes under sterile conditions (Todd, 1991), but infection with *Schistosoma* inhibits diabetes development (Cooke *et al.*, 1999). This result is further borne out by induction of a Th2 shift in the NOD mice treated with *Schistosoma* egg antigen prior to oral administration of the insulin B chain antigen (Maron *et al.*, 1998). In contrast, the presence of *Syphacia obvelata* can alter the immune response of nude mice and trigger the development of lymphoma (Beattie *et al.*, 1980). These findings prompted Gale (2002) to suggest that pinworms in humans were able to modulate immune responses to the point of inhibiting the progression of immune-mediated diseases such as asthma and diabetes, though he did not rule out the existence of competing elements in the environment.

One issue certainly neglected by many workers is the level of chemical molecules released into the environment that have entered the food chain in recent years. For example in Sardinia, DDT, used in massive doses in the malaria eradication campaign conducted by the Rockefeller Foundation in the 1950s to exterminate *Anopheles labranchiae* (Logan, 1953), continues to be detected in the food chain.

Another variable for Sardinia concerns the incidence of non-HIV-associated Kaposi's sarcoma (KS) (classic/Mediterranean and African), which varies enormously from one country to another. In Africa it accounts for 3–9% of all malignant tumours, against 0.02% in the USA. This pattern appears to depend on local human herpes virus 8 (HHV-8) infection levels. In Italy, sero-prevalence data indicate marked geographical variability and can be directly correlated with incidence (Calabro *et al.*, 1998; Whitby *et al.*, 1998; Santarelli *et al.*, 2001). The highest incidence rates have been recorded in north Sardinia. (Cottoni *et al.*, 1996; Serraino *et al.*, 1998) and in Sicily (Franceschi & Geddes 1995). The aetiology of KS remains unclear, but analysis of data collected to date suggests that genetic factors, cell-mediated immunodeficiency, immunological disorders, HHV-8 infection, virus–host interaction and a series of environmental and other risk co-factors (McHardy *et al.*, 1984; Geddes *et al.*, 1995; Ziegler *et al.*, 1997; Iscovich *et al.*, 2000) play a role (yet to be hierarchically defined) in triggering this disease. Kaposi's sarcoma appears to be caused by a complex interaction between HHV-8, the infected host and micro and macro-environmental co-factors. The localized distribution of HHV- 8, its peculiar zoning, the transmission pathways and conditions for primary infection and KS expression as well as the pathogenetic significance of the risk co-factors still need to be elucidated.

Even though KS is not familial, a certain genetic predisposition does appear to be demonstrated by the more frequent occurrence of certain HLA haplotypes in patients affected with KS compared to the population as a whole (Contu *et al.*, 1984). The importance of cellular immuno-deficiency has been demonstrated by studies on HIV positive subjects and by the possible regression in immunodepressed patients when they revert to normal immune conditions. It has recently been suggested that blood-feeding arthropods are involved in HHV-8 infection and KS pathogenesis (Ascoli *et al.*, 2002), whereas the relation with malaria does not appear to be a risk factor (McHardy *et al.*, 1984; Cottoni *et al.*, 1997).

On the other hand, it is not known whether the gradual 'disappearance' of helminths from the Mediterranean population (or developed countries) is one of the potential causes promoting the development of HHV-8 and KS expression as a result. All these considerations prompted us (we too are not immune to scientific fashion) to explore the causal relationship!

Though sufficient data are available on the disease-promoting immunomodulatory properties of helminths, the same cannot be said of the beneficial immunomodulatory properties of worm infection. In fact these beneficial effects are contradicted by other data which suggest that a dominant Th2 response can render the host more susceptible to other clinically more important diseases and can also affect the host's ability to produce a continuous protective immune response (Bentwich *et al.*, 1995, 1999). Helminths contribute to the spread of such diseases as HIV infection and TB (Bentwich *et al.*, 1995, 1999) and in these cases it is not the host but the unrelated pathogen that benefits from parasite-induced immune malfunction. Further data substantiating this hypothesis are provided by the improvement achieved by an Ethiopian Jewish community on emigration to Israel: a change in their life style coincided with a standard of immune response comparable with that typical of people living in developed countries (Bentwich *et al.*, 1999). Perhaps all this simply demonstrates that the world's poorest populations, often undernourished and with very poor standards of living, are exposed to cumulative health insults to which helminths contribute only in part.

No conclusive evidence exists for either of these hypotheses but it would appear more reasonable to think in terms of a complex interaction that opens up new opportunities in parasitology for increasingly interdisciplinary and valuable research.

https://doi.org/10.1079/JOH2003176 Published online by Cambridge University Press

Acknowledgements

We thank Marco Songini and Anna Casu for providing useful information and helpful discussion during the preparation of this article. This work was supported by grants from the Assessorato all'Igiene e Sanità, Regione Autonoma della Sardegna.

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(Accepted 10 March 2003)