**Giardia comes of age: progress in epidemiology, immunology and chemotherapy**


Although giardia was probably the first microorganism to be seen by the human eye during the second half of the seventeenth century (Dobell, 1920) progress in our understanding of the biology of the organism and the pathogenesis of human disease has been slow. Indeed the organism was forgotten for two hundred years until Vilem Lambl rediscovered the parasite in 1885 producing the first microscopic drawings of the trophozoite and cyst (Dobell, 1942). The question as to whether giardia was a pathogen or commensal was debated for many decades during the first part of the twentieth century (Fantham & Porter, 1916). There is however, now irrefutable evidence that the parasite is a cause of both acute and chronic diarrhoea and may produce retardation of growth and development in infants and young children (Farthing, 1989).

Detailed investigation of the biology of the organism did not begin until the late 1970s when human isolates of giardia were first established in axenic culture (Meyer, 1976). During the last fifteen years there have been important developments in our understanding of the molecular epidemiology of giardiasis, the pathogenesis of diarrhoeal disease (Katekari & Farthing, 1992), the contributions of the immune system to clearance of infection and the development of protective immunity (Farthing, 1990) and some early clues to the mechanisms of treatment failure.

Giardia is a curious organism having two nuclei but a complete lack of intracellular organelles, including the notable absence of mitochondria. It has become apparent that the Giardia genus probably has a unique place in evolution since it may represent the missing link between prokaryotes and eukaryotes (Kabnick & Peattie, 1991). Analysis of the Giardia lamblia 16S-like rRNA sequence has shown it to be unusually rich in G + C content (75%) and has only 1453 nucleotide positions, features which are more typical of prokaryotes than of eukaryotes (Sogin *et al*., 1989). Comparison with a variety of other eukaryote and prokaryote small sub-unit rRNAs indicates that the Giardia genus represents the earliest diverging lineage in the eukaryotic line of descent. We have studied codon usage by *G. lamblia* studying 3135 codons from eight sequenced genes and can confirm that the pattern more closely resembles that of archaebacteria than of eukaryotes (Char & Farthing, 1992).

Giardia rose to fame during the 1960s on the wave of a series of waterborne epidemics in the USA and Europe (Craun, 1984; Jephcott, Begg & Baker, 1986). These emphasized the importance of sedimentation and filtration of municipal water supplies in addition to chlorination, to which giardia cysts are relatively insensitive. It also became apparent in the USA that a variety of wild animal species, particularly the beaver, carried giardia cysts and it was suggested that these animals formed an important and largely inaccessible reservoir of human giardia (Davies & Hibler, 1979). Recent studies in domestic pets and ruminants indicate that there are other potential sources and reservoirs of giardia close to man (Winsland *et al*., 1989; Buret *et al*., 1990). Although, as yet, giardiasis has not been unequivocally shown to be a zoonosis, phenotypic and genotypic identity has been shown between animal and human isolates which would strongly support this view (De Jongh, Majewska & Kasprzak, 1990). The extraordinary clinical diversity seen in patients with giardiasis has never been explained satisfactorily. Worldwide the majority of infected individuals have few or no symptoms. However, giardia can produce severe acute diarrhoea which in a proportion of individuals may persist and be associated with overt malabsorption (Farthing, 1989). Weight loss can be profound and in children this may result in impaired growth and development (Farthing *et al*., 1986). This spectrum of clinical response could be attributed to variation in virulence of giardia isolates, to host factors such as the immune response or to a combination of the two. There is now firm evidence that morphologically identical human giardia isolates do vary with respect to their surface antigens, isoenzyme profiles, susceptibility to proteases (Smith *et al*., 1982; Andrews *et al*., 1989; Nash *et al*., 1990; Nash, Merritt & Conrad, 1991).
and genetically as revealed by restriction fragment length polymorphism analysis and DNA fingerprinting (Nash et al., 1985; Meloni, Lymbery & Thompson, 1989; Upcroft, Mitchell & Boreham, 1990; Carnaby, McHugh & Farthing, 1991). Preliminary results suggest that differences in phenotype and genotype may correlate with virulence in experimental models of infection (Aggarwal & Nash, 1987; Nash et al., 1987; Cevallos et al., 1991; Cevallos & Farthing, 1992). However, specific virulence factors have not been identified although with the development of potential typing systems and in-vivo and in-vitro test systems to distinguish relatively virulent from avirulent isolates, it should not be long before candidates begin to emerge. An intact immune system is important for eradicating the infection and for the development of protective immunity (Farthing, 1990). Giardia is essentially a luminal parasite and thus the study of immune mechanisms has focused on antibody production, namely secretory IgA. Studies in experimental animal models confirms the importance of slgA in protecting against infection although there have been relatively few studies in humans. Recent work however, suggests that failure to develop an IgA response to a 57 kDa parasite antigen, which is one of giardia's heat shock proteins, is associated with persistent infection despite an IgG response to this antigen and IgA responses to other giardia polypeptides (Char et al., 1991, 1992).

Effective antimicrobial chemotherapy for giardiasis came in the 1940s with mepacrine (Thomas, 1952). However, mepacrine fell from favour with the availability of metronidazole (Darbon et al., 1962) and the succession of nitroimidazole derivatives that appeared subsequently. Metronidazole or tinidazole given over three days or as a single dose, respectively, can be expected to be successful in approximately 90% of cases (Mendelson, 1980). Metronidazole however, is not approved by the Food and Drug Administration in the USA for the treatment of giardiasis, although used widely by physicians for that purpose. Nitroimidazole derivatives are generally thought to act by their action on nitro anion radical metabolites binding to DNA or proteins, although metronidazole or its radicals have a rapid inhibitory effect on respiration in giardia trophozoites (Paget et al., 1989) suggesting an action on an enzyme of the respiratory pathway. Treatment failures however, do occur with nitroimidazole derivatives (Mendelson, 1980), drug resistance being one possible explanation. Following the development of drug sensitivity assays (Boreham, Phillips & Shepherd, 1984; Inge & Farthing, 1987) it has been possible to show that human isolates vary in their susceptibility to this class of drugs (Boreham, Phillips & Shepherd, 1987; McIntyre et al., 1986). Drug resistance in giardia is associated with decreased uptake of metronidazole in vitro (Boreham, Phillips & Shepherd, 1988) which may well be related to the negative correlation which exists between metronidazole resistance and pyruvate: ferredoxin oxidoreductase activity (Smith, Bryant and Boreham, 1988).

Recent work indicates that human giardia infection can be 'mixed' containing isolates of different phenotype and genotype (Carnaby et al., 1991). Treatment with a nitroimidazole derivative may permit emergence of a relatively resistant isolate within a 'mixed' infection. Treatment failure with a nitroimidazole derivative presents clinicians with a problem since other therapeutic options are limited. Giardia are sensitive to the antimalarial drug mepacrine and also furazolidone, but these are not always readily available. Preliminary clinical studies suggest that mebendazole is effective at high dose in patients with giardiasis (Al-Waili, Al-Waili & Saloon, 1988) although this claim has been contested subsequently (Gascon et al., 1990). The related compound, albendazole has also been assessed in an uncontrolled study and may have some activity against Giardia spp. (Meloni et al., 1990). General use of these drugs in giardiasis cannot be recommended until further controlled clinical trials have been performed. In-vitro studies show that giardia is susceptible to a variety of other antimicrobial chemotherapeutic agents including chloroquine, pyrimethamine, mefloquine, azithromycin, doxycycline and rifampicin (Gordts et al., 1985; Crouch, Seow & Thong, 1986). Dyadic combinations of some of these drugs were synergic in vitro and might be worthy of trial in resistant cases (Crouch et al., 1990). Indeed combination therapy with mepacrine and metronidazole has been shown to be of value in human infections refractory to treatment with a single agent. There have been a number of minor sorties to search for novel approaches to anti-toxoplasmodial chemotherapy. The tricyclic antidepressant drug, clomipramine inhibits growth of Giardia lamblia in vitro, possibly through its ability to inhibit the membrane-associated ATPase (Weinbach, Costa & Wieder, 1985). D-propranolol, a membrane-stabilizing drug inhibited both motility and...
growth of giardia in vitro and a clinical report in a case of metronidazole failure suggests that addition of DL-propranolol was associated with rapid eradication of the parasite (Popovic & Milovic, 1990). It remains to be established whether such approaches will be confirmed to be of clinical value.

Thus, despite rapid advances in our understanding of the biology of giardia many deficits remain. Our concepts of the pathogenesis of diarrhoeas are incomplete and there is a need for new, effective antigiardial drugs to deal with treatment failures and the emerging problem of nitroimidazole resistance.

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References

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Optimal dosage of β-lactam antibiotics: time above the MIC and inoculum effect


New β-lactam antibiotics are usually administered in similar dosage regimens to older agents. In the treatment of severe infection, an adult may receive between 6 to 12 g (some-