Fatal infection by two genera of free-living amoebae, *Acanthamoeba* and *Naegleria*, is well documented, with numerous human cases recorded. The first cases were published as early as 1965 in Australia (Fowler & Carter, 1965), and since then from all over the world (Ma et al., 1990). *Acanthamoeba* causes granulomatous amoebic encephalitis (GAE), almost invariably in the immunocompromised (Khan, 2006), but this amoeba more commonly causes amoebic keratitis (AK), a sight-threatening eye infection associated with corneal trauma and/or contact-lens use (Niederkorn et al., 1999). A single species of *Naegleria*, *Naegleria fowleri*, also produces an encephalitis in humans, and this is known as primary amoebic meningoencephalitis (PAM). Like GAE, PAM is almost always fatal (Barnett et al., 1991). More recently, a third genus of amoeba, *Balamuthia*, has been discovered to cause a fatal encephalitis in humans (Visvesvara et al., 1990). This encephalitis is known as *Balamuthia* amoebic encephalitis (BAE). At present there is a single species in this novel genus, *Balamuthia mandrillaris*, and all isolates from animals and humans have been ascribed to this single species. There are worrying features of BAE that are emerging, even compared to GAE and PAM. PAM is restricted to bodies of warm freshwater, such as swimming pools and lakes, and so can be avoided after its presence has been identified. An example of this is the geothermal baths of the city of Bath in England (Kilvington et al., 1991). GAE is a disease of the immunocompromised, and so affects a small subpopulation of individuals who could conceivably be monitored for early signs of GAE; for example, by inspection of cerebrospinal fluid (CSF) (Deetz et al., 2003). Present data indicate that BAE is more difficult to detect, as it is sporadic, affecting both immunocompromised and immunocompetent, otherwise healthy, individuals with little evidence of predisposing factors (see below). The unpredictable nature of the disease may mean that BAE is even less likely to be diagnosed in time for medical intervention and, like GAE and PAM, it is essential for BAE to be diagnosed early if it is to be treated successfully (Petry et al., 2006). Worryingly, BAE may be a relatively common amoebal encephalitis (Schuster et al., 2004), and some cases reported to be due to *Acanthamoeba* (Matson et al., 1988) have subsequently been shown to be due to *B. mandrillaris* (Deetz et al., 2003).

*B. mandrillaris* was first described as a ‘Leptomyxid’ amoeba due to its polyaxial body shape (Visvesvara et al., 1990). Later genetic analysis, however, revealed that *Balamuthia* is a close relative of *Acanthamoeba* (Amaral Zettler et al., 2000; Booton et al., 2003) and *Protoanomamoeba* (Dykova et al., 2005). Despite this relationship, *B. mandrillaris* is found to prey on *Acanthamoeba* (Schuster et al., 2003; Matin et al., 2006a), but not on a range of bacteria offered, as the vast majority of amoeboid organisms do (Matin et al., 2006a). This explains why *B. mandrillaris* has only been isolated from the environment on two occasions to date (Dunnebacke et al., 2003; Schuster et al., 2003), as standard methods involve inoculating material from the environment onto lawns of bacteria (usually *Escherichia coli*), allowing amoebae to crawl out, feeding on the bacteria as they do so.

Like GAE, BAE has often been reported to occur subsequent to a skin lesion (Deetz et al., 2003; Deol et al., 2000), in particular the face (Pritzker et al., 2004; Seas & Bravo, 2006; Valverde et al., 2006). *B. mandrillaris* amoebae spread from this primary lesion through the blood, and then penetrate the brain—brain barrier to gain access to the brain. *Naegleria fowleri*, on the other hand, is thought to gain access to the brain in a relatively direct manner. This amoeba finds its way onto the nasal mucosa during swimming in warm water, digests its way up through the olfactory nerve and into the brain (Cabanes et al., 2001). This may be why the course of infection due to *N. fowleri* is so rapid. Both GAE and BAE share the features of haematogenous spread from an often chronic primary lesion, and this may explain the long time-course of the infection relative to PAM. The existence of this phase of infection prior to access to the brain may also offer a target for intervention. There are several possible reasons why so many pathogens and facultative pathogens end up in the brain. *Acanthamoeba*, *Naegleria* and *Balamuthia* are all obligate aerobes and the brain is well oxygenated. The brain also excludes antibodies and other components of the immune system. A simpler explanation is that when these organisms do penetrate the brain, their presence becomes very obvious.

A large Californian study looking at causes of encephalitis found at least 13 939 cases of acute encephalitis diagnosed between 1990 and 1999 (Trevejo, 2004). Amongst these cases, 0.1% were attributed to *Naegleria*, 0.63% to other protozoans, and 34.7% were from unspecified causes. The California Encephalitis Project (CEP) (Glaser et al., 2003; Schuster et al., 2006), covering a similar time span, 1998–2000, identified three fatal cases from the 334 patients who met the criteria for CEP and whose encephalitis had been caused by *Balamuthia* (Schuster et al., 2004). No cases of encephalitis caused by either *Acanthamoeba* or *Naegleria* were seen within the CEP population, but immunocompromised patients were excluded from the study (Schuster et al., 2006), which would account for the lack of *Acanthamoeba* cases. Prima facie, these data would suggest that in the state of California in the 1990s, *Balamuthia* and *Naegleria* each accounted for approximately 0.1% of total encephalitis cases in the otherwise healthy population!

From the limited data currently available we can conclude that BAE occurs in healthy people of any age (perhaps with emphasis on the very young and old), with a bias toward males, and a reported bias toward Hispanic people. The Hispanic bias (Schuster et al., 2004) is difficult to understand, since *B. mandrillaris* infects such a broad range of mammals in addition...
to humans, but it has been reported that Hispanic peoples are less able to make antibodies against certain Acanthamoeba species (Chappell et al., 2001). Another possibility is that Hispanics in Southern California are more likely than other groups to be exposed to infected soils during agricultural activities (Schuster et al., 2004).

Temperature seems to be an important factor in the occurrence of BAE, as the disease seems to be more common in warmer regions, such as Southern California and South America (Seas & Bravo, 2006). Isolates of Balamuthia can grow at 37°C in vitro, so perhaps this amoeba can only survive in warm soils? Certainly the ability to grow at 37°C is a prerequisite for human pathogenicity.

The immunization of mice by Acanthamoeba has been demonstrated to have a protective effect (Rowan-Kelly & Ferrante, 1984), and the role of antibodies in GAE is generally accepted (Cursons et al., 1980; Ferrante, 1991). Many individuals have detectable antibody titres against B. mandrillaris (Schuster et al., 2006), and while these levels are not as high as those of individuals infected with the amoeba, this may indicate prior exposure to B. mandrillaris or a cross-reactivity with other organisms. However, antibodies specific for B. mandrillaris are known not to recognize Acanthamoeba and vice versa (Huang et al., 1999; Schuster et al., 2006), and so, unfortunately, harmless exposure to the related Acanthamoeba does not naturally immunize against B. mandrillaris. The fact that some patients have an elevated titre of antibodies to B. mandrillaris and yet still succumb to the infection demonstrates that the situation is complex.

In this issue of the journal, Matin et al. (2007) shed new light on this antibody paradox. These authors show that normal human serum has antibodies that recognize B. mandrillaris. These normal sera inhibit the growth of B. mandrillaris in culture and have a demonstrable amoebicidal activity. These findings are in accordance with recent reports that many non-infected individuals possess antibodies against B. mandrillaris (Schuster et al., 2001). The serum also inhibits the ability of the amoeba to bind to human brain microvascular endothelial cells, which form the blood–brain barrier. These observations are consistent with the suspected course of a typical BAE progression. The infection begins with a skin lesion, during which the amoeba penetrates the body. The amoeba presumably grows at a rate that is limited by the antibodies in the serum. This serum not only holds the amoeba population in check but possibly prevents amoebae from binding to the endothelia of the blood–brain barrier, and may neutralize toxins such as the proteases (Matin et al., 2006b) that are presumed to take part in the destruction of the cells. The chronic phase of the infection is likely to be at the stage where the blood–brain barrier is intact, but as soon as the amoeba breaks through into the brain, the amoebae, being free of antibody, are able to multiply and to destroy the brain rapidly. Matin et al. (2007) also show that there is a remarkable consistency in the subgroup of about 10 amoebal proteins recognized by these human sera. Further work is necessary to determine if these may be usefully targeted for immune therapy, such as inhibiting the proteases that break down the blood–brain barrier, or by direct amoebicidal activity.

Despite these advances, we remain fundamentally ignorant about this organism and the disease that it causes. Several questions remain unanswered.

Is this amoeba really free-living? It has been isolated from soil on two occasions, so does this mean that it is to be found in virtually all soils, like its cousin and presumed food source Acanthamoeba? Is there a necessary third party?

How many people are at risk? Are Hispanic peoples actually more prone to this disease and, if so, why?

Is there a geographical limit to the distribution, or is B. mandrillaris, like Acanthamoeba, practically ubiquitous?

Are there other members of the Balamuthia genus and, if so, what threat do they represent?

The estimated frequency data from the various Californian studies (and others) are likely to be an underestimate (Schuster et al., 2006), and so it is important that amoebae, especially Balamuthia and Naegleria, should be considered as possible causes of encephalitis when confronted with patients displaying general encephalitis symptoms. Further work, such as that described in this issue (Matin et al., 2007), is crucial to the understanding of B. mandrillaris pathology if therapies are to be developed. It is also crucial that further advances are made in the detection of amoebal encephalitis cases (Qvarnstrom et al., 2006).

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