parasite would do well to limit parasite protein release and peptide migration across the PVM.

The inverse correlation was supposed to convey the intuitively reasonable concept that a parasite that only just survives in an inappropriate host is an easy target for immune attack. This correlation is unlikely to be an absolute phenomenon as many factors are involved, such as nonspecific immunity, which can play a crucial role in irradiated sporozoite immunity in rats and mice (D. Mazier, pers. commun.). Nevertheless, in support I would argue that when referring to infectivity in this context I am referring to infectivity of only the liver. A/J mice are, for example, excellent hosts for the blood stage forms and OFA albino rats (the only rat strain for which I can find comparative data) are 6.35-fold less efficient at supporting the EE stage of *P. berghei* than are *Thamnomys* livers[3]. C57 black mice are good hosts for the EE stage[4] of *P. berghei* and are much more difficult to protect with irradiated sporozoites than are BALB/c mice[5]. I agree that *P. yoelii* infectivity is more similar to the situation in humans (50% infective dose of 8–40 sporozoites[5] compared with 100% with ten sporozoites for *P. vivax*[6]) but mice are harder to protect with *P. yoelii* sporozoites. In no mouse strain could 100% protection be achieved with a single dose; few C57/10 mice, for example, were protected even after 4–6 doses. Ungureanu *et al.* suggest that the reason for the difference may be the much higher viability of dissected *P. yoelii* sporozoites when compared with *P. berghei* sporozoites[5]; injection of nonviable parasites may help induce protection (perhaps via spleen-derived interferon and/or intrahepatic death). The low infectivity of laboratory-grown NF54 *P. falciparum* sporozoites to humans[6] might suggest that human vaccination with these sporozoites might also be aided by low viability. The inverse correlation holds in at least one case for *P. yoelii*, in that BALB/c mice are less susceptible and easier to protect than C57/BL6 mice (D. Mazier, pers. commun.).

Despite the higher infectivity of *P. yoelii* in mice, there is circumstantial evidence suggesting that EE stages of *P. yoelii* are considerably less well supported by mouse hepatocytes than by *Thamnomys* hepatocytes[7].

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Human Filariasis

The Endemic Normal in Lymphatic Filariasis: A Static Concept

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Residents of areas endemic for lymphatic filariasis are continually exposed to infection with mosquito-transmitted infective larvae (L3), some of which survive to become adult worms and subsequently produce microfilarial (mf) transmission stages. The question of whether naturally acquired resistance occurs in adult residents of endemic areas has recently become of interest as the development of molecular vaccines against filarial parasites is being considered[1,2]*. There have been two epidemiological approaches to demonstrate acquired resistance to filariasis in human populations. In this review Karen Day examines both approaches in the context of an immunoepidemiological study of bancroftian filariasis in Papua New Guinea (PNG). The merits of each as a conceptual framework for studies of protective immunity in lymphatic filariasis will be discussed.*

One approach to defining acquired resistance to lymphatic filariasis in human populations has been to divide adult residents of an endemic area into three categories: (1) individuals with no detectable mf and no symptoms or history of disease despite life-long exposure to infection; (2) asymptomatic microfilarial carriers and (3) individuals with filarial disease who may or may not be microfilarial carriers. The infection status of the amicrofilaremic individuals in the first category who have been called endemic normals is unclear. They are arbitrarily defined in a point prevalence survey by the sensitivity of the diagnostic test used to screen for mf with no knowledge of previous parasitological history. They most likely represent a heterogeneous group of individuals ranging from those who are truly free of adult worms to those who have single-sex infections, few adult worms or occult infections[1,2]. Despite the lack of knowledge concerning the parasitological status of the endemic normal, these individuals have been presumed to be resistant to infection with L3, whereas microfilaremic adults have been considered to be susceptible. In fact, the assumed state of resistance of the endemic normal has become an entrenched concept in the filariasis

Table 1. PC-Ag levels in relation to mf density in the PNG study population[a]

Mf class (mf ml^{-1})	Number of subjects	Mean PC-Ag level[b]
0	56	4.1 ± 0.1
1–10	78	4.2 ± 0.1
>1000	116	9.6 ± 0.2

[a] PC-Ag, phosphorylcholine-containing antigen; mf, microfilarial.
[b] PC-Ag levels were calculated by immunoradiometric assay as described previously[5,15].

literature[1,2], even though there has only been one published study[3] in lymphatic filariasis that has defined the parasitological status of endemic normals using sufficiently sensitive diagnostic techniques.

Analysis of amicrofilaremic adult residents of Mauke, Cook Islands, identified only seven of a population of 459 as candidate endemic normals[3]. These individuals appeared to have a distinct immunological response to a 43 kDa larval antigen compared to age-matched microfilaremic controls. Differential recognition of this immune response by infected, compared to uninfected, individuals has promoted the endemic normal as an epidemiological model to identify correlates of protective immunity[1,3].

The Dynamics of Lymphatic Filariasis

An alternative approach to defining acquired resistance has been to consider the observed worm burden in an individual as a dynamic process. This worm burden must arise as a balance between the infection rate of the host with L3, some of which survive to become adult worms, and the mortality pattern of these adult worms[4,5]. Regulation of worm burden may occur by the mechanism of concomitant immunity. Such immunity would be characterized by the gradual development of resistance to reinfection with L3 in the continuing presence of a long-lived, fecund adult worm population. Unlike schistosomiasis[6–9], the concept of concomitant immunity has been little explored in human filariasis. This is somewhat surprising, given that there is clear evidence of acquired resistance in trickle infection experiments of *Brugia pahangi*-infected cats, an important animal model for human filariasis[10,11]. Cats trickle infected with L3 become resistant to reinfection and this resistance is directed mainly at incoming larvae, with the majority being destroyed within 24 h of injection. Interestingly, adult worms in these cats are relatively long lived (life expectancy of approximately two years) and arise mainly from early infections, when hosts are essentially naïve. Maizels and Lawrence[12] have argued that adult worms and mf in such infections induce a state of immunological tolerance that prevents elimination of these life cycle stages. They believe that this mechanism of tolerance does not influence the development of anti-L3 immunity and is consistent with the existence of concomitant immunity.

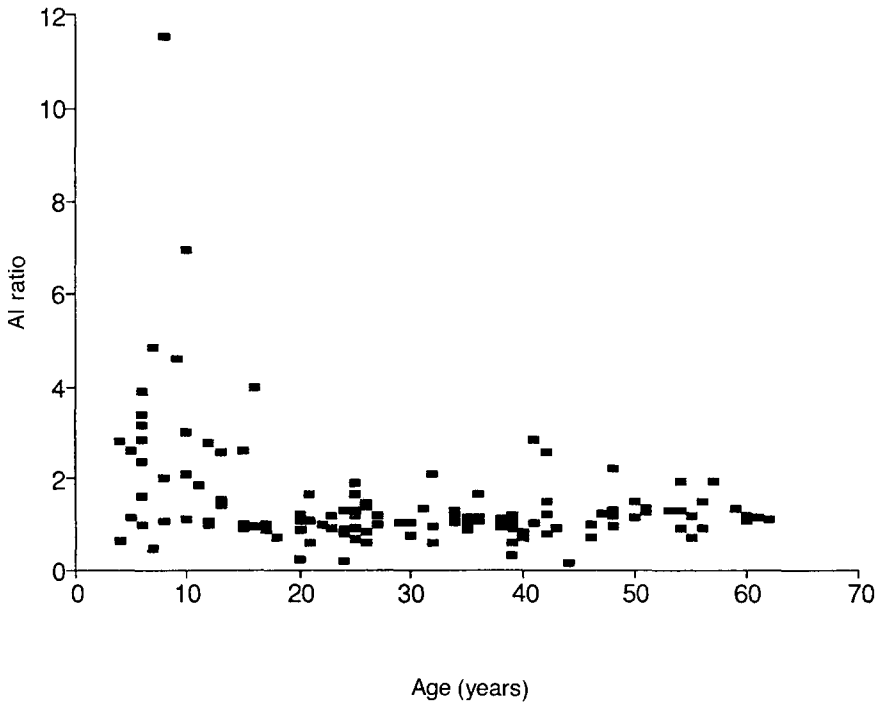

Fig. 1. Age-specific ratio of phosphorylcholine-containing antigen (PC-Ag) levels, described as antigen index values (AI) (Refs 5,15) for 126 residents of the bancroftian filariasis endemic area of Dreikikir, PNG. The AI ratio was calculated as the PC-Ag levels (determined by immunoradiometric assay) at two time points 12 months apart. It is an indirect measurement of the dynamics of worm burdens in this 12-month period.

In helminth research, the classic epidemiological approach to demonstrate acquired resistance has been to examine reinfection intensities and rates after chemotherapy with a drug that kills adult worms[7,8]. Such epidemiological studies of reinfection with *Schistosoma mansoni*[7] and *S. haematobium*[8], which controlled for individual exposure to infection, have provided strong evidence that humans acquire resistance to reinfection with increasing age and that this resistance is immune mediated[9]. Similar age-structured population studies of reinfection rates after chemotherapy have not been attempted in humans with lymphatic filariasis for a number of reasons. Diethylcarbamazine (DEC), the drug that is currently available for treatment in humans, is only partially macrofilaricidal[13], making reinfection data difficult to interpret. Long-term experiments are required to evaluate reinfection and it is also difficult to control for individual variation in exposure to L3. In the absence of reinfection data in lymphatic filariasis, an alternative approach has been to examine the age-specific dynamics of infection[4,5].

The Endemic Normal in PNG

Intense transmission of *Wuchereria bancrofti* infection occurs in the East Sepik Province of PNG, as evidenced by infection occurring at an early age and high overall microfilarial rates and densities[14,15]. In order to test the null hypothesis concerning the endemic normal in PNG, ie. that all adult residents of this endemic area are infected, the infection status of amicrofilaremic asymptomatic adults was assessed in greater detail by testing clinical response to DEC as well as by two serological tests. Both circulating phosphorylcholine-containing antigen (PC-Ag) and filarial-specific IgG4 levels have been shown previously to correlate with active *W. bancrofti* infection and mf density in this population[15,16]. It is assumed that both serological measurements must be related to adult worm burden in some density-dependent manner[15]. From a village of 92 adult residents, only five (5.4%) were found to be candidate endemic normals, ie. amicrofilaremic and asymptomatic, when 2-ml night blood samples were examined on two occasions at 12-month intervals. All five amicrofilaremic adults had circulating 200 kDa PC-Ag, a specific indicator of active *W. bancrofti* infection[15] and three out of the five had systemic and/or localized side reactions

to DEC. All had detectable levels of filarial-specific IgG4. Using these criteria of active infection, there were no worm-free endemic normals in this PNG population where intense transmission of infection occurs.

Analysis of PC-Ag levels in relation to mf densities for adult residents of this endemic area showed that individuals with zero and low mf (1–10 mf ml^{-1} blood) had similar PC-Ag levels compared to individuals with high mf (>1000 mf ml^{-1} blood) (see Table 1). As PC-Ag levels appear to be an indicator of mature worm burdens[15], these data imply that individuals with zero mf have similar worm burdens to those with low mf.

Population Dynamics in PNG

A longitudinal study of the dynamics of *W. bancrofti* infection in the above-mentioned PNG population, using changes in levels of circulating PC-Ag as an indirect measure of adult worm burden, showed that worm burdens were observed to increase in children and adolescents but not in adults over a 12-month period (Fig. 1 and Ref. 5). Consideration of the PNG data in the context of a simple mathematical model describing the dynamics of adult worm populations in relation to their life expectancy and attrition of larvae during establishment showed that the observed patterns of change of intensity of infection with age were consistent with the acquisition of resistance to new infections with increasing experience of infection[5]. Furthermore, this analysis indicated that this resistance was likely to be directed at the early larval stages and was independent of existing adult worm burden. When the age-specific dynamics of the serological response to the L3 surface was examined in this population, a correlation was established between age and prevalence of antibody. Resistant adults but not children had detectable anti-L3 surface responses irrespective of current worm burden[17].

Conclusion

Immunoepidemiological studies from PNG have shown that all adult residents of this bancroftian filariasis-endemic area were infected. Inability to find endemic normals in PNG reflects the sensitivity of the measurements to define infection as well as the intensity of transmission in this endemic area rather than unique genetic and behavioural features of the PNG study population. This study and that reported previously[3] indicate that, if you look carefully enough in any infected population, the endemic normal is a rare individual.

Consideration of worm burdens and mf density as a function of PC-Ag level in the PNG population highlighted two features of endemic normal studies. First, endemic normal studies[3] have focussed on individuals in the zero and high mf groups as resistant and susceptible, and ignored the resistance status of the large proportion of the population within the low mf class. Second, comparison of amicrofilaremic adults with microfilaremic adults in any study population essentially examines these two groups as a function of worm burden.

Analyses of the dynamics of *W. bancrofti* infection from PNG[5] have shown that the rate of gain of infection levelled off after reaching adulthood. A similar study of the dynamics of *W. bancrofti* microfilaremia from Pondicherry in India showed that the rate of gain of infection declined in adults but the rate of loss of infection was age independent[4]. These infection dynamics in PNG and Pondicherry populations strongly suggest that resistance to re-infection is acquired with increasing experience of infection, ie. age, and resistance status is not reflected by current worm burden. They are consistent with the hypothesis that concomitant immunity develops in the majority of the adult population after repeated exposure to infection and that immunity must be directed at early larval stage antigens rather than adult antigens. If this hypothesis is correct, the identification of mechanisms of protective immunity and candidate vaccine antigens will require age-stratified analyses[17] to compare adults and pre-immune children, analogous to acquired immunity studies in human schistosomiasis[7–9].

In contrast, the static concept of the endemic normal implies a state of perfection or complete immunity rather than the dynamic process of regulation of worm burden by attrition of the larvae. It considers current worm burden rather than experience of infection, ie. age, as the important variable in the definition of resistance status and it only identifies a minority of the adult population as resistant. The concept of the endemic normal therefore excludes the existence of concomitant immunity. It may also confuse the identification of protective antigens and immune responses, if anti-L3 and anti-adult immunity operate independently in an age-dependent manner, as suggested by measurement of anti-L3 surface antibodies[17].

On the basis of the recent population dynamic studies[4,5], I would argue that the static concept of the endemic normal be replaced by the hypothesis that concomitant immunity exists in the majority of the adult population in filariasis-endemic areas. This hypothesis is well founded in helminth biology[6] and is supported by trickle infection experiments in *B. pahangi*-infected cats[10,12]. Concomitant immunity provides a realistic conceptual framework in which to examine mechanisms of protective immunity in lymphatic filariasis.

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