PARASITE BIOLOGY AND BIOCHEMISTRY

REPRODUCTIVE BIOLOGY AND POPULATION DYNAMICS OF *ONCHOCERCA VOLVULUS* IN THE VERTEBRATE HOST

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KEYWORDS : *Onchocerca volvulus*, worm load, fecundity, turnover of microfilariae.

In filariases microfilariae play the key role for the transmission of the parasite and the development of pathology. Therefore, various aspects of the parasites' reproductive biology and population dynamics in the host are prime considerations for the control of filariases. Since microfilariae released from female worms are not discharged, but accumulate and survive in the host for a considerable time, the reproduction of filarial worms is suggested to be regulated to a greater degree by intrinsic host factors. These may, however, vary during the course of infection and depend on the response of the individual host. Since there is no pulmonary microfilarial reservoir in *Onchocerca* infections, different regulatory mechanisms are suggested for species with blood- and skin-dwelling microfilariae (Schulz-Key, 1988).

DEVELOPMENT AND HABITAT OF ADULT WORMS

Infective larvae (L3) moult to the fourth stage within 3-7 days, and a final moult to be the juvenile worms occurs several weeks later. Immature worms seem to be attracted to existing nodules, may settle on their surface and form satellite or composite nodules. Thus, young, old and calcified dead worms are often found associated in the same nodule (Schulz-Key, 1988).

Onchocercomata are found on distinct sites of predilection of the host and their accumulation may help the sexes to find each other. On average, 1-2 male and 2-3 female worms can be found distributed in 80 % of the nodules. Accumulation of more than 50 worms can occur, but this is the exception. In contrast to the sessile females male worms regularly leave the nodules – presumably in search of females. Consequently, in excised onchocercomata a striking predominance of female worms can be observed owing to male worms just migrating in the host. The wandering impulsion and thus the reproductive activity of male worms is suggested to decrease with age (Karam *et al.*, 1987). Most male worms in overaged worm populations, e.g. as found in the late phase of the Onchocerciasis Control programme (OCP) in West Africa, are inactive and separated from non gravid females although associated in the same nodule.

MATING, INSEMINATION AND FERTILIZATION

The stimuli and pheromons which attract males to the females in the nodules are unknown. Since spermatozoa are scanty in females with empty uteri, shedding of oocytes into the uteri seems to be a prerequisite for the sti-

mulation of males to mate. Conversely, oocytes are released from the narrow ovaries into the wide lumen of the uteri independent from the presence of male worms. In case female worms do not mate, large numbers of degenerating and shrinking oocytes may accumulate which are later resolved. The development of an oocyte to a mature microfilariae is estimated to take not more than three to four weeks (Schulz-Key and Karam, 1986). Fecundity starts after a pre-mature period of nine to twelve months (Duke, 1980 ; Schulz-Key, 1988 ; Soboslay *et al.*, 1991).

DISTRIBUTION OF EMBRYONIC STAGES IN FEMALE WORMS

Mature female worms of species with blood-dwelling microfilariae were found to contain embryonic stages and microfilariae in both branches of the uteri throughout the patent infection (Mössinger and Wenk, 1986 ; Mössinger and Barthold, 1987). Their embryograms showed rather homogeneous patterns indicating that uterine microfilariae were permanently present and continuously released into the host reflecting a rapid turnover of the microfilarial population.

In comparison, female *O. volvulus* show a more heterogeneous distribution of uterine stages. Less than two third of the adult females contain embryonic stages and microfilariae, although primary oocytes are present attached to the rachis in the distal ovaries in all of them. One third, or in overaged worm populations distinctly more, show oocytes or empty uteri, even in big nodules when associated with a sufficient number of males. This typical distribution pattern of varying reproductive phases is independent from the age of the worms and the adult worm load.

The phase of transient reproductive "inactivity" seems to be rather short. Oocytes and early developmental stages of a new batch can be observed in female worms which still harbour some degenerating microfilariae, but no further precursor stages of the latter. In addition, the analysis of worms isolated from patients treated with drugs that interfere with the intrauterine development lead to the conclusion that the reproduction of *O. volvulus* occurs in asynchronous batches lasting two to four months each (Schulz-Key and Karam, 1987). Similar reproduction patterns can be observed for *Onchocerca ochengi* (Trees *et al.*, 1992), *Onchocerca gibsoni* (Vankan and Copeman, 1988), *Onchocerca* species in wild animals (Schulz-Key, 1983). They might be typical for species with skin-dwelling microfilariae and might play a role in the microfilarial population dynamics in the host.

REPRODUCTIVE CAPACITY AND MICROFILARIAL RELEASE IN VIVO

The reproductive capacity of female worms can be assessed by embryograms which quantify the number of intrauterine stages actually present. This "snapshot" can-

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not, however, indicate how many microfilariae per day are produced or released. Observations on worms maintained *in vitro* or excised from patients at different periods after chemotherapy suggest that about 700 to 1,500 microfilariae per female are released into the host on average per day, i.e. only a portion of the microfilariae actually developed *in utero* leave the female worms (Schulz-Key, 1990). The daily output is an order to magnitude lower than that observed for species with blood-dwelling microfilariae. For example, females of *Loa*, *Litomosoides* or *Dirofilaria* expel 10,000 to 20,000 microfilariae into the host daily (Eberhard and Orihel, 1988 ; Hawking, 1954 ; Mössinger and Wenk, 1986 ; Weinstein and Sawyer, 1961).

In contrast to other filarial species microfilariae of *O. volvulus* are not expelled by the female worm but leave it actively one by one. It takes at least 5 to 10 seconds for a microfilaria to leave the female worm when it has arrived at the vulva. This is obviously a limiting factor. Microfilariae which stay in the uteri gradually degenerate and are later on resorbed (Schulz-Key, 1988).

THE REPRODUCTIVE LIFESPAN OF *O. VOLVULUS*

The reproductive lifespan has been estimated by longitudinal skin snip surveys during 13 to 14 years of successful vector control in the OCP area in West Africa (Plaisier *et al.*, 1991). It was concluded that the reproductive lifespan of the savanna strain of the parasite lies between 9 and 11 years. This indirect assessment of the reproductive lifespan is in accordance with observations made by direct analysis of onchocercomata from onchocerciasis patients in other areas of the control programme (Karam *et al.*, 1986).

REGULATION OF REPRODUCTION

The mechanisms that regulate microfilarial production and release are unknown and seem to be multifactorial. It is rather unlikely that the release of microfilariae is mechanically regulated by the female worms, e.g. by the sphincter of the vulva. The unpaired part of the uterus may be empty or sparsely populated, even when sufficient heavily wriggling microfilariae are present in a posterior section of the uterus, perhaps in expectance of a stimulus for active evasion. Observations on patients treated with ivermectin on very old worm populations in areas with interrupted transmission support our hypothesis that the production and release of intra-uterine microfilariae are partially or even predominantly regulated by rather independent mechanisms, some of them intrinsic in the host (Schulz-Key, 1990).

In overaged worm populations, e.g. in inhabitants of the OCP area, the turnover of microfilariae in the host is very low. An increased number of female worms show non-fertilized oocytes or empty uteri, and in gravid females the number of development uterine stages is low, many of them pathologically altered. A long lasting interruption of transmission provided gradual changes of the immune response of the host at the same time.

The observed decrease of parasite reproductivity seems to be reversible (Awadzi *et al.*, 1985), and therefore some of

these changes might be correlated with the regulation of the parasitdermia. Interestingly, uterine microfilariae produced in worms of inhabitants who have already become negative in skin snips are no more released and degenerate in hypergravid female worms (Schulz-Key *et al.*, 1987). This observation further supports our hypothesis of independent mechanisms of microfilaria production and release.

SKIN MICROFILARIAE AND ADULT WORM LOAD

The correlation between the adult and microfilaria load is varying from person to person depending on the response of the individual host. There are several indications that both the accumulation of adult *O. volvulus* in the host and the microfilarial densities are not linearly established. There seems to be an increasing resistance to superinfections, as has been already suggested for some other filarial infections (Barthold and Wenk, 1992 ; Day *et al.*, 1991), and microfilarial densities are controlled by the host's immune system as well. These are major restrictions for any calculation and estimation.

In a hyperendemic village of the Liberian rain-forest, the micro- and macrofilarial loads were assessed by skin snips and nodulectomies (Albiez *et al.*, 1984). All palpable nodules of 117 inhabitants were removed, the worm loads analysed and correlated with the mean microfilarial load, which was 12×10^6 microfilaria in each patient on average (Schulz-Key, 1990). A mean lifespan of a microfilaria of 1.0 to 1.5 years anticipated a stable parasitdermia needs to replace $22\text{--}33 \times 10^3$ microfilariae every day. Based on the above estimated daily release of microfilariae approximately 30 females are needed to produce these numbers of microfilariae. Although an unknown proportion of deep-laying nodules was not accessible for extirpation, our assessment of a mean number of 16 females (geom. mean) per patient found in the excised nodules signals a right order to magnitude in our calculation.

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ISOLATION OF NEW MARKERS TO DETECT GENETIC VARIATION IN *ONCHOCERCA VOLVULUS*

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KEYWORDS : *Onchocerca volvulus*. genetic variability. RAPD. microsatellite. PCR.

SUMMARY

Newly developed techniques (RADP : Random Amplified Polymorphic DNA and microsatellite DNA sequences) were used to study genetic

variation in *Onchocerca volvulus*. RAPD technique, derived from the Polymerase Chain Reaction (PCR), allow clear distinction between the different species of the genus *Onchocerca*.

Microsatellite DNA has been shown to be useful as polymorphic markers for populations and even for individuals. Short repeated sequences (CA repeats) have been isolated, and a PCR assay using such microsatellite DNA sequences to generate polymorphisms is currently being experimented.

In West Africa, particularly in the OCP area (Onchocerciasis Control Programme), at least two strains of the parasite are known to exist. The so-called "savannah" strain associated with high blindness level, and the "forest" strain associated with a milder form of onchocerciasis which causes blindness in less than 1 % of the population (Prost *et al.*, 1980). However, Sierra Leone is exceptional in that high levels of blindness are found in forest areas (McMahon *et al.*, 1986).

Various *Onchocerca* specific DNA probes have been developed (Meredith *et al.*, 1989) and also strain specific probes (Erttmann *et al.*, 1987 and 1990). These DNA probes sequences are based on the 150 bp repeat family. Recently, studies conducted in West Africa have shown that DNA probe classification correlates with the epidemiology of blindness (Zimmerman *et al.*, 1992).

In Cameroon, the situation is more complex, notably the pathology of blindness is distributed without apparent relation to bioclimatic zones (Duke, 1981 ; Boussinesq *et al.*, 1993). To date, no studies similar to those carried out in West Africa have been done in Cameroon.

We are investigating other molecular markers that may be useful for studies on genetic variation in *O. volvulus*, namely RAPD and microsatellite sequences.

Polymorphism in genomic fingerprints generated by Random Amplified Polymorphic DNA (RAPD) is a recently developed assay that is based on the amplification by the Polymerase Chain Reaction (PCR) of random DNA fragments. In RAPD mapping, decamer oligonucleotide primers of arbitrary sequence but with a GC content of 50 % or higher are used to amplify fragments of genomic DNA (Williams *et al.*, 1990 ; Welsh and McClelland, 1990).

RAPD has been successfully applied to the analysis of genomic DNA variation of several organisms (Williams *et al.*, 1990 ; Hadrys *et al.*, 1992 ; Klein-Lankhorst *et al.*, 1991 ; Crowhurst *et al.*, 1991 ; Mazurier *et al.*, 1992). Strains can be distinguished by comparing polymorphisms in genomic fingerprints (Welsh *et al.*, 1991).

The particular advantages of RAPD technology is that small amounts (nanograms) of genomic DNA are needed and, unlike PCR, no prior sequence knowledge is needed.

Microsatellite DNA sequences are short (from 2 to 5 bp), tandemly repeated sequences that have been shown to be useful as polymorphic markers for populations and even for individuals. They have been reported from a large panel of eukaryotic species such as human, mouse, cattle, whales and insects (Tautz, 1989 ; Love *et al.*, 1990 ; Swaiger *et al.*, 1992 ; Weber and May, 1989 ; Hughes and Queller, 1993). These dinucleotide repeats are referred to as microsatellites (Litt and Luty, 1989). Most of these regions are less than 200 bp in length and PCR is used to detect length polymorphisms.

We describe here the use of the RAPD assay for the detection of variation in the genus *Onchocerca*, using a set of 20

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