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REVIEW

The histopathology of bancroftian filariasis revisited: the role of the adult worm in the lymphatic-vessel disease

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Although morphology is generally limited to static images, the histopathological features of bancroftian lymphatic disease are presented here in a way that is as dynamic as possible and closely associated with the clinical, ultrasonographic and surgical characteristics. The protean spectrum of alterations seen in the host's lymphatic vessels is discussed, and the changes caused by the live and dead worms are highlighted, as independent events. Evidence of a remodelling process, in which the lymphatic endothelial cells appear to have a key role, is provided for the first time. Despite many new pieces of information, there remain many 'blank pages' in the natural history of bancroftian filariasis.

Since the parasites that cause it occur in an estimated 120 million people, lymphatic filariasis (LF) should no longer be considered an 'exotic' disease (Michael *et al.*, 1996). As in other emerging and re-emerging infections, studies on the pathology of LF, both in laboratory animal models and in humans, are becoming increasingly important in supporting other, epidemiological, clinical and pathogenetic investigations (Shwatz, 1997). Apart from a few investigations in Puerto Rico (O'Connor *et al.*, 1930;

O'Connor, 1932*a, b*; O'Connor and Hulse, 1935), the western world showed little interest in LF until the Second World War, when American soldiers in the South Pacific developed the disease. The infections in the United States Army triggered several histopathological studies (Hartz, 1944; Wartman, 1944; Michael, 1945; Rifkin and Thompson, 1945). The results supported the idea that only the dead, adult worms were able to cause the tissue alterations that eventually led to obstruction of the lymphatic vessels. In endemic areas, however, it soon became clear that the pathology of bancroftian filariasis (the disease, caused by *Wuchereria bancrofti*, accounting for >90% of LF cases) encompasses distinct histological features

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related to the presence of both live and dead parasites (Lichtenberg, 1957; Lichtenberg and Medina, 1957; Galindo *et al.*, 1962), as extensively documented in reports based on local surgical pathology (Jungmann *et al.*, 1991, 1992). It has long been known that filarial infection can lead to a very wide range of clinical manifestations (Bancroft, 1878). Those carrying the adult worms may be microfilaraemic or amicrofilaraemic, and be asymptomatic or have clinical lymphangiectasia, filarial acute lymphangitis (FAL), the chronic manifestations of the disease (lymphadenopathy, lymphoedema, haematuria, hydrocele, chylocele and chyluria) and/or tropical pulmonary eosinophilia (TPE) (Dreyer *et al.*, 1998b).

Excellent reviews on the pathogenesis of filariasis, as followed by histopathology, already exist (Connor *et al.*, 1986; Lichtenberg, 1987). The main aims of the present review were to integrate the results of clinical, surgical, ultrasonographic and histopathological observations made in Brazil, and so provide support for the dynamic model recently proposed for lymphatic-vessel disease in bancroftian filariasis (Dreyer *et al.*, 2000). The main postulate of this model is that, given sufficient time, all of those who carry adult *W. bancrofti* will develop localized lymphangiectasia in the vicinity of worm 'nests' (here defined as groups of living adult parasites detectable, by ultrasonography or peri-operatively, in a given segment of lymphatic vessel).

In general, human infection with *W. bancrofti* remains asymptomatic until at least one intervening factor overwhelms the compensatory capabilities of the lymphatic system. Different sets of compounding factors contribute to the development of the different types of chronic, lymphatic filarial disease. These factors may be permissive (such as very heavy worm burdens) or non-permissive [such as host responses and antifilarial treatment with diethylcarbamazine (DEC)]. Bacterial infections play an important role in the development of lymphoedema and elephantiasis (Dreyer *et al.*, 2000).

TISSUE ALTERATIONS RELATED TO THE LIVE ADULT PARASITES

In the surgical material investigated in Brazil, from patients who live permanently in endemic areas, all alterations seen in the host tissue appear to be attributable to the mature, adult *W. bancrofti*. The most common change in the presence of living worms (and the earliest in the dynamic model) is subclinical lymphangiectasia of variable degree, which can be observed *in vivo* even in childhood (Dreyer *et al.*, 1999b). In such lymphangiectasia, the vessels containing structurally intact worms are distended, without any inflammatory response in the wall [Fig. 1(a)]. Lymphocytes, a normal component of the lymph, may be occasionally found in the unusually wide lumens, and sometimes lining the endothelial layer. In the walls of the affected vessels, areas of fibrosis alternate with scant and isolated smooth muscle cells, and areas with muscle-cell hyperplasia. The endothelial cells are preserved but, under electron microscopy, show bulging nuclei and many pinocytotic vesicles; abundant collagen bundles, aligned in different orientations, can also be seen. It should be emphasised that such lymphangiectasia can be observed in conducting vessels as well as in the afferent, efferent and hilar vessels of lymph nodes (Jungmann *et al.*, 1991, 1992). However, living parasites do not seem to occur in lymph-node parenchyma. They have never been observed in such tissue and their dimensions and very active, serpentine movements — the so-called filaria dance sign (FDS), which is relatively easy to detect by ultrasonography (Amaral *et al.*, 1994) — make it unlikely that they have been missed.

The nearer the parasite is to the lymph node the greater the level of lymphoid hyperplasia, characterized by large follicles with germinal centres, hypercellularity of the paracortical areas, and sinus histiocytosis. However, unless the parasite is present within the adjacent lymphatics, the histopathological picture is non-specific.

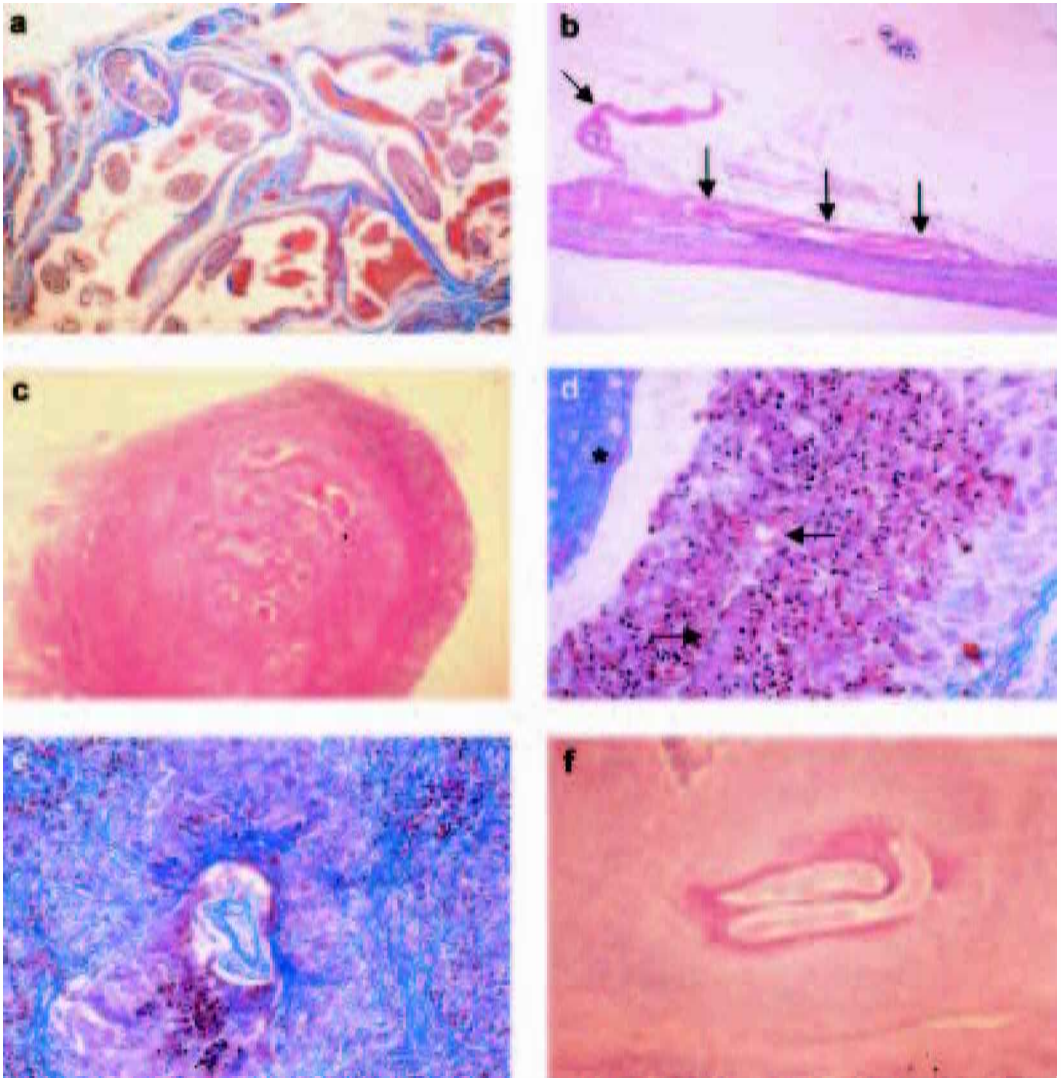


FIG. 1. Sections of biopsy samples from Brazilian patients infected with *Wuchereria bancrofti*. (a) Lymphatic vessels with distended lumens harboring living, female adult worms, and without any inflammatory reaction in the wall. (b) Degenerated worm connected to the lymphatic-vessel wall by strands of fibrin-like material; the lumen is still open and there is mild parietal infiltration by inflammatory cells. (c) Filarial lymphangitis: the lymphatic vessel is occluded by granulomatous inflammatory reaction around dead, adult worms. (d) Area of the filarial granuloma showing degenerated worm (○) surrounded by cellular debris, eosinophils and large macrophages; a detached segment of the cuticle (→) can be seen among eosinophils. (e) Filarial granuloma around a disintegrating adult worm, composed of eosinophils, large macrophages, lymphocytes and plasma cells. (f) Filarial granuloma exhibiting the Splendore-Hoeppli reaction around a dead, adult worm.

All the available evidence indicates that lymphatic dilation, rather than being immunologically mediated, is caused by factors related to the parasites themselves. It can be observed in nude or SCID mice

infected with *Brugia* species (Vincent *et al.*, 1984; Nelson *et al.*, 1991), for example, and can be reversed in nude mice by removing or killing the adult worms (Vickery *et al.*, 1983, 1991). Ultrasonographic investigations

have revealed that the anatomical location of a given nest containing live adult worms changes very little over time, and that active translocation of adult worms is therefore limited despite their vigorous in-vivo thrashing (Amaral *et al.*, 1994; Dreyer *et al.*, 1995, 1996a, 1998a; Norões *et al.*, 1997). That lymphangiectasia is not restricted to the exact segment where a nest is located indicates that it may be the result of the action of soluble parasite-specific factors or excretory-secretory products on responsive lymphatic endothelial cells. Such cells probably have a prominent role in the pathogenesis of lymphangiectasia. Strategically located, at the interface between the parasite and its host, they are both a target for and a source of many active substances, serving as a sensory layer that can assess lymphodynamic, humoral and cellular signals (Vane *et al.*, 1990; Mantovani *et al.*, 1992; Gibbons and Szau, 1994).

TISSUE ALTERATIONS RELATED TO DEAD ADULT PARASITES

Although the adult worms probably die in a broad variety of circumstances — depending on parasite- and host-related factors or on the action of drugs such as DEC — the pathways leading to their death are not perfectly understood. Whatever the cause, only the death of adult worms elicits a local inflammatory reaction (Jungmann *et al.*, 1991, 1992; Figueredo-Silva *et al.*, 1996). Thus, lymphangiectasia and the inflammatory reactions are two independent components of lymphatic pathology that are apparently triggered, respectively, by 'toxins' from the living, adult worms and by the reactions of the host to damaged or dead worms. In many subjects, the non-DEC-triggered death of adult worms merely results in the subclinical formation of nodules that are only detected, incidentally, during physical examinations; in other subjects, an episode of acute filarial lymphangitis (AFL) develops (Olszewski *et al.*, 1993; Dreyer

et al., 1999a). The characteristic AFL syndrome can be readily differentiated from non-filarial acute dermatolymphangioadenitis (ADLA) — an infection of the skin associated with secondary inflammation, in the capillary and draining lymphatics and the regional lymph nodes, caused by pyogenic bacteria (Olszewski *et al.*, 1993; Dreyer *et al.*, 1999a). Unfortunately, the old term used to describe any acute inflammatory episode in patients living in areas where filariasis was endemic, adenolymphangitis (ADL), covers both AFL and ADLA.

Following DEC treatment of infected males, nodules develop in the lymphatics of the scrotal area (in exactly the same site where the cessation of adult-worm movements can be detected by ultrasonography); this corresponds to the inflammatory reaction to the dead parasite. Little is known, however, about the early modifications in the worms or the human tissues that follow parasite death. It is reasonable to presume that parasites stop moving and then become coiled as they die. When live and actively moving *W. bancrofti* are removed from lymphatics during surgery they immediately become coiled and immobile, only uncoiling and beginning to thrash about again when put into a physiological solution. Use of fixatives, such as formalin and glutaraldehyde, on the coiled worms make this phenomenon irreversible. If the worms do coil tightly as they die this may explain why, in spite of the length of the parasite, the diameter of the post-treatment nodules rarely exceeds 1 cm. In contrast to the live parasites, which are always found completely free inside the lumen of a lymphatic vessel, damaged worms sometimes appear connected to the vascular wall by strands of fibrin-like material [Fig. 1(b)]. In this very early phase, the inflammatory reaction is mild, non-specific and parietal. It gradually increases until the inflammatory exudate fills the vascular lumen [Fig. 1(c)]. Lethally injured adult worms appear deformed, their external membranes rupture, and fragments of cuticle can be seen within clusters of eosinophils [Fig. 1(d)]. As

the process of adult-worm disruption progresses, each parasite probably splits into several fragments, each with its own, local cluster of inflammatory reaction. At this stage, the inflammatory reaction is clearly granulomatous, with variable numbers of eosinophils, lymphocytes, plasma cells and large macrophages [Fig. 1(e)]. Rarely, neutrophils may accumulate in the centre of the granuloma, in a sort of 'micro-abscess'. There may be large numbers of eosinophils, especially in cases treated with DEC, and hyalinized eosinophilic material (the Splendore-Hoeppli reaction) is frequently seen [Fig. 1(f)]. This is in sharp contrast with the absence of eosinophils in tissue where live worms are found, even in individuals with unrelated peripheral-blood hyper-eosinophilia (Figueredo-Silva *et al.*, 1994). Eosinophil peroxidase, major basic protein [Fig. 2(a)], and eosinophil cationic protein can be identified around the dead parasites, indicating that eosinophil degranulation has occurred. Studies on the tissue deposition of eosinophil proteins in filarial infections are limited to the identification of major basic eosinophil protein around the microfilariae of *Onchocerca volvulus* in patients treated with DEC (Kephart *et al.*, 1984); it is assumed that eosinophils are capable of destroying microfilariae. Electronic microscopy of lymph nodes from patients with onchocerciasis who had been treated with DEC revealed that most of the microfilariae were in states of severe degeneration, and that eosinophils were stuck to each microfilaria, forming an exact mould of its surface (Racz *et al.*, 1982). The Mazzotti reaction, observed in onchocerciasis cases after treatment with DEC, is considered to be a result of eosinophil degranulation (Ackerman *et al.*, 1990). In schistosomiasis, eosinophils are thought to be capable of killing schistosomula (Butterworth *et al.*, 1979; Jong *et al.*, 1984; Ackerman *et al.*, 1985), the results of cryofracture studies indicating that the eosinophils liberate their granules directly on the parasitic surface (Caufield *et al.*, 1980a, b). Within 5 h of eosinophils adhering to schistosomula, damage

to the tegument of the parasites allows the eosinophils to attack the underlying layers (Glauert *et al.*, 1987). Interestingly, eosinophils co-cultured with endothelial cells kill more antibody-coated schistosomula than eosinophils that have been freshly isolated (Rothenberg *et al.*, 1987). There is, as yet, no direct evidence that eosinophils can kill adult *W. bancrofti* (which brings to mind the restraint of Gulliver by the Lilliputians in *Gulliver's Travels*) but they do seem to play an important role in the disruption of dead or damaged worms.

In the later reaction to a dead parasite, multinucleated giant cells are seen in close contact with the remnants of the parasite, and concentric deposition of collagen takes place [Fig. 2(b)]. Besides the granulomatous response around the worms, a diffuse infiltration of mononuclear cells, eosinophils and fibroblasts, permeated by a great number of newly formed blood capillaries, is observed in the bordering areas, the lymphatic wall becoming barely discernible [Fig. 2(c)]. In fact, this is true granulation tissue, with two putative functions: to conduct recruited inflammatory cells to granulomas; and, especially, to facilitate the remodelling process (see below). In segments of the lymphatic vessel adjacent to the nodule around a dead parasite, a non-specific, chronic inflammation of the vessel wall is also observed.

It has been claimed that 'allergens' released into the circulation by the adult female parasites, as they periodically release microfilariae, provoke the AFL episodes (Kar *et al.*, 1993). If so, the site of AFL attacks should be unrelated to the location of the adult worms, and the frequency of the episodes should exhibit some pattern consistent with the biology of parturition in filarial worms. However, spontaneous and DEC-triggered episodes of AFL virtually always occur where adult worms can be located by ultrasound and thus constitute a localized host reaction. While the adult worm remains alive, microfilariae can be found in the lymph nodes of untreated patients without

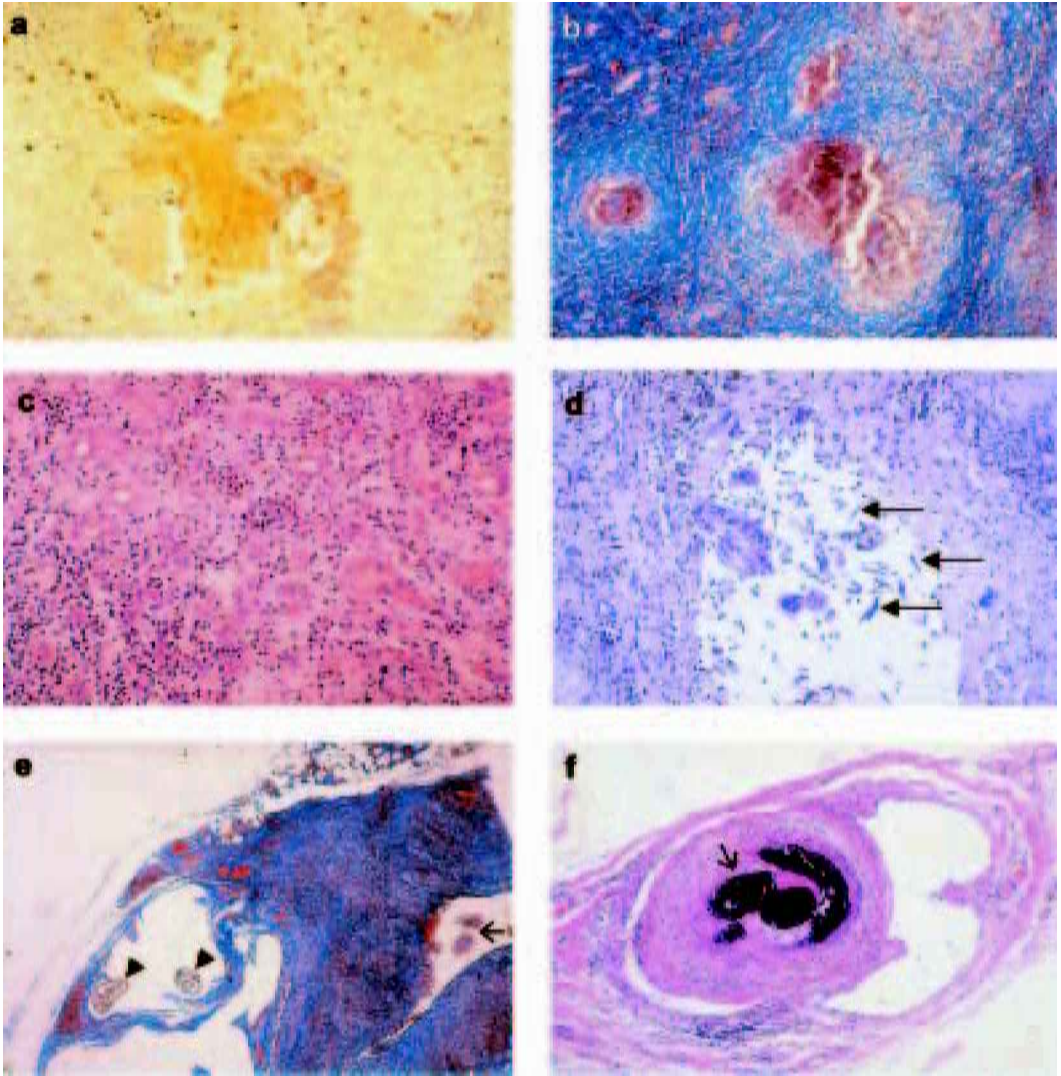


FIG. 2. Sections of biopsy samples from Brazilian patients infected with *Wuchereria bancrofti*. (a) The results of using the indirect peroxidase method to reveal eosinophilic major basic protein in the centre of a granuloma around a degrading adult worm; intact eosinophils are also seen at the periphery. (b) Concentric deposition of collagen in old filarial granulomas around almost completely disintegrated, adult worms. (c) Granulation tissue in the areas around filarial granulomas and extending to the lymphatic wall and adjacent tissues. (d) Segment of a lymphatic vessel proximal to the granulomatous reaction to a degrading adult worm, showing microfilariiae, some of which are entrapped in small polypoid projections of the endothelial surface. The vessel wall is infiltrated by mononuclear inflammatory cells. (e) Concomitant presence of viable (\blacktriangle) and degenerating (\leftarrow) adult worms in the same tissue section. The absence of inflammation is noticeable in the lymphatic vessel containing the live worm, in contrast to the thickened, inflamed wall of the vessel holding the degenerated parasite. (f) Old, fibrotic granuloma centred on the calcified residues (\leftarrow) of an adult worm. The lymphatic lumen is patent and the inflammatory infiltrate is minimal and focal.

any concomitant inflammation (Figueredo-Silva *et al.*, 1994). Thus, parturition should be distinguished from the non-physiological

release of microfilariae as the wall of the adult worm ruptures post-mortem; in the latter event, damaged microfilariae are seen

entrapped in fibrin-like material or attached to the endothelial surface, in this case appearing to contribute to the inflammatory reaction [Fig. 2(d)].

Almost all of the many hypotheses advanced on the pathogenesis of LF are based on the assumption that the pathology observed is the result of the cell-mediated and/or humoral responses of the host (Ottesen, 1992; Freedman, 1998). The results of investigations using animal models have indicated that the hosts' systemic immune responses are the key triggering events in the death of the parasites and the development of tissue alterations (Klei *et al.*, 1981, 1982, 1990; Vickery *et al.*, 1983, 1991; Lawrence, 1996). If this were the case, it might be expected that all the adult worms present in any single host would be at similar levels of attack and degradation at any one time. Curiously, however, at least in infections with Brazilian *W. bancrofti*, dead and living, apparently normal, adult worms may be present in one host at the same time. The two types of worm may be in different lymphatic ducts [Fig. 2(e)] or even in the same nest (Norões *et al.*, 1997). There may be a mixed reaction to DEC treatment, for example, in which not all parasitic nests, and even not all parasites in the same nest, are destroyed (Norões *et al.*, 1997). Although the relevance of the immune response in the host-parasite relationship of LF seems beyond dispute, the cause-effect linkage between the host's systemic immune response and the pathogenesis still remains under-explored. As stated by Piessens *et al.* (1990) more than a decade ago, 'the mere fact that filarial parasites develop and survive in immunocompetent hosts indicates that they are able to evade or resist host immune responses, either by hampering immune effector mechanisms or by preventing the development of immune responses with protective potential'. The concurrent presence of dead, dying and apparently healthy adult worms in the same host may indicate that some but not all parasites have lost the 'art of surviving'. Among the humans who live in endemic

areas, clinically detectable disease may be the expression of a mechanism that is the exception, rather than the rule, and host response may be a reaction to, rather than the cause of, the death of the adult worms. Inasmuch as the core of parasitism is a long-standing, undisturbed co-existence, *W. bancrofti* infection may represent a unique model of evolutionary sophistication in host-parasite interplay. The adult parasite's death is not obligatorily caused by the host's immune mechanisms, and factors intrinsic to the parasites themselves, such as age and metabolic disturbance, the disappearance of intracellular 'bacteria' from the tissues of the filarial nematode (Taylor *et al.*, 1999) or the action of drugs, must be considered. Furthermore, the various developmental stages of the parasite appear to vary in their susceptibility to the various immune-effector mechanisms. During tropical pulmonary eosinophilia, for example — a syndrome caused by immune hypersensitivity to microfilariae — living adult worms can be visualized by ultrasound (Dreyer *et al.*, 1996b).

THE REMODELLING PHASE

Under ideal conditions, the outcome of the host's inflammatory response is the elimination of the source of tissue insult and the restoration of normal architecture and function. In LF, the period in which the adult worm, once dead, remains recognizable in human tissues and the time-course of its complete absorption are unknown. Nothing, theoretically, prevents the abnormal lymphatic vessels returning to the condition they were in during the lymphangiectasia (in the early stages of the pathogenesis) once the adult worms are dead and completely degraded. However, some additional degree of local, residual damage may be expected. Calcification of the degenerating worms (but not of the lymphatic wall) was described in the reports of the first histopathological studies (O'Connor *et al.*, 1930; Wartman, 1944; Michael, 1945) and is commonly

observed. This certainly constitutes a significant factor in the termination of the inflammatory response, reducing or even abolishing the source of parasite components. It is an unpredictable phenomenon, affecting some parasites and sparing others, even in the same individual. In onchocerciasis, the calcified material (unlike the calcium phosphate deposited in human necrotic tissues) is predominantly calcium carbonate, calcification occurs quickly (within 4 weeks in experimental studies), and is related primarily to high percentages of old worms and, to a lesser extent, to increasing age of the human host (Albiez, 1985). In the more advanced stages of human LF, the inflammatory reaction gradually loses strength and the granuloma seems to shrink to a wall-like, fibrotic protuberance, eventually allowing the lymphatic lumen to re-open [Fig. 2(f)]. In thrombosed lymphatic vessels there may be recanalization of the lumen, renewing at least some flow in the occluded vessels. These alterations, also observed in animal models (Crandall *et al.*, 1987; Snowden and Hammerberg, 1989; Case *et al.*, 1992), seriously contest the premise that permanent occlusion of lymphatic vessels leads to the chronic syndromes seen in bancroftian filariasis. However, increased susceptibility to local bacterial infections may develop in human LF, as it does in some experimental models (Bosworth and Ewert, 1975), and sequential adult-worm death with incomplete healing of the primary anatomical lesion can modify the histopathological features and clinical course of filarial disease.

The sequential alterations seen in lymphatic-vessel architecture during the course of bancroftian filariasis, from lymphangiectasia to the development and decline of granulomatous inflammatory response and eventually to re-opening and/or recanalization, are remodelling events. 'Vascular remodeling is an active process of structural alteration that involves changes in at least four cellular processes — cell growth, cell death, cell migration, and production or degradation of extracellular matrix — and is dependent on a

dynamic interaction between locally generated growth factors, vasoactive substances, and hemodynamic stimuli' (Gibbons and Szau, 1994). Applied originally to blood vessels, this concept has been extended to lymphatic vasculature, as detailed in the recent and comprehensive review by Witte *et al.* (1997). In bancroftian filariasis, endothelial cells appear to be key players in the remodelling process, although there has been little relevant research on their role.

The absence of an effective animal model for bancroftian filariasis has led many scientists to work with *Brugia* species, generating extensive and important experimental data of great relevance to clinicians. Clearly, closer co-operation between basic and medical researchers must be encouraged if all the available data and resources for future research are to be used most effectively.

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REFERENCES

- Ackerman, S. J., Gleich, G. J., Loegering, D. A., Richardson, B. A. & Butterworth, A. E. (1985). Comparative toxicity of purified human eosinophil granule cationic proteins for schistosomula of *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene*, **34**, 735–745.
- Ackerman, S. J., Kephart, G. M., Francis, H., Awadzi, K., Gleich, G. J. & Ottesen, E. A. (1990). Eosinophil degranulation. An immunologic determinant in the pathogenesis of the Mazzotti reaction in human onchocerciasis. *Journal of Immunology*, **144**, 3961–3969.
- Albiez, E. J. (1985). Calcification in adult *Onchocerca volvulus*. *Tropical Medicine and Parasitology*, **36**, 180–181.
- Amaral, F., Dreyer, G., Figueredo-Silva, J., Norões, J., Cavalcanti, A., Samico, S. F., Santos, A. & Coutinho, A. (1994). Live adult worms detected by

- ultrasonography in human bancroftian filariasis. *American Journal of Tropical Medicine and Hygiene*, **50**, 753–757.
- Bancroft, J. (1878). Cases of filarious disease. *Transactions of the Pathological Society of London*, **29**, 407–419.
- Bosworth, W. & Ewert, A. (1975). The effect of *Streptococcus* on the persistence of *Brugia malayi* and on the production of elephantiasis in cats. *International Journal for Parasitology*, **5**, 583–589.
- Butterworth, A. E., Wasson, D. L., Gleich, G. J., Loegering, D. A. & David, J. R. (1979). Damage to schistosomula of *Schistosoma mansoni* induced directly by eosinophil major basic protein. *Journal of Immunology*, **122**, 221–229.
- Case, T. C., Unger, E., Bernas, M. J., Witte, M. H., Witte, C. L., McNeill, G., Crandall, C. & Crandall, R. (1992). Lymphatic imaging in experimental filariasis using magnetic resonance. *Investigative Radiology*, **27**, 293–297.
- Caufield, J. P., Korman, G., Butterworth, A. E., Hogan, M. & David, J. R. (1980a). The adherence of human neutrophils and eosinophils to schistosomula: evidence for membrane fusion between cells and parasites. *Journal of Cell Biology*, **86**, 46–63.
- Caufield, J. P., Korman, G., Butterworth, A. E., Hogan, M. & David, J. R. (1980b). Partial and complete detachment of neutrophils and eosinophils from schistosomula: evidence for the establishment of continuity between a fused and normal parasite membrane. *Journal of Cell Biology*, **86**, 64–76.
- Connor, D. H., Palmieri, J. R. & Gibson, D. W. (1986). Pathogenesis of lymphatic filariasis in man. *Zeitschrift für Parasitenkunde*, **72**, 13–28.
- Crandall, R. B., Crandall, C. A., Hines, A. S. & Nayar, N. K. (1987). Peripheral lymphedema in ferrets infected with *Brugia malayi*. *American Journal of Tropical Medicine and Hygiene*, **37**, 138–142.
- Dreyer, G., Amaral, F., Norões, J. & Medeiros, Z. (1995). Ultrasonographic evidence for stability of adult worm location in bancroftian filariasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **88**, 558.
- Dreyer, G., Addiss, D., Norões, J., Amaral, F., Rocha, A. & Coutinho, A. (1996a). Ultrasonographic assessment of the adulticidal efficacy of repeated high-dose ivermectin in bancroftian filariasis. *Tropical Medicine and International Health*, **1**, 427–432.
- Dreyer, G., Norões, J., Rocha, A. & Addiss, D. (1996b). Detection of living adult *Wuchereria bancrofti* in a patient with tropical pulmonary eosinophilia. *Brazilian Journal of Medical and Biological Research*, **29**, 1005–1008.
- Dreyer, G., Addiss, D., Santos, A., Figueredo-Silva, J. & Norões, J. (1998a). Direct assessment *in vivo* of the efficacy of combined single-dose ivermectin and diethylcarbamazine against adult *Wuchereria bancrofti*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **92**, 219–222.
- Dreyer, G., Figueredo-Silva, J., Neafie, R. C. & Addiss, D. G. (1998b). Lymphatic filariasis. In *Pathology of Emerging Infections 2*, eds Nelson, A. M. & Horsburgh Jr, C. R. pp. 317–342. Washington: ASM Press.
- Dreyer, G., Medeiros, Z., Netto, M. J., Leal, N. C., de Castro, L. G. & Piessens, W. F. (1999a). Acute attacks in the extremities of persons living in an area endemic for bancroftian filariasis: differentiation of two syndromes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **93**, 413–417.
- Dreyer, G., Norões, J., Addiss, D., Santos, A., Medeiros, Z. & Figueredo-Silva, J. (1999b). Bancroftian filariasis in a paediatric population: an ultrasonographic study. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **93**, 633–636.
- Dreyer, G., Norões, J., Figueredo-Silva, J. & Piessens, W. F. (2000). Pathogenesis of lymphatic disease in bancroftian filariasis. A clinical perspective. *Parasitology Today*, **16**, 544–548.
- Figueredo-Silva, J., Dreyer, G., Guimarães, K., Brandt, C. & Medeiros, Z. (1994). Bancroftian lymphadenopathy: absence of eosinophils in tissues despite peripheral blood hyper eosinophilia. *Journal of Tropical Medicine and Hygiene*, **97**, 55–59.
- Figueredo-Silva, J., Jungmann, P., Norões, J., Piessens, W. F., Coutinho, C., Brito, C., Rocha, A. & Dreyer, G. (1996). Histological evidence for the adulticidal effect of low doses of diethylcarbamazine in bancroftian filariasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **90**, 192–194.
- Freedman, D. O. (1998). Immune dynamics in the pathogenesis of human lymphatic filariasis. *Parasitology Today*, **16**, 229–234.
- Galindo, L., Lichtenberg, F. & Baldizón, C. (1962). Bancroftian filariasis in Puerto Rico: infection pattern and tissue lesions. *American Journal of Tropical Medicine and Hygiene*, **11**, 739–748.
- Gibbons, G. H. & Szau, V. J. (1994). The emerging concept of vascular remodeling. *New England Journal of Medicine*, **330**, 1431–1438.
- Glauert, A., Butterworth, A. E., Sturrock, R. F. & Houba, V. (1987). The mechanism of antibody-dependent, eosinophil-mediated damage to schistosomula of *Schistosoma mansoni in vitro*: a study by phase-contrast and electron microscopy. *Journal of Cell Science*, **34**, 173–192.
- Hartz, P. H. (1944). Contribution to the histopathology of filariasis. *American Journal of Clinical Pathology*, **14**, 34–43.
- Jong, E. C., Chi, E. Y. & Klebenoff, S. J. (1984). Human neutrophil-mediated killing of schistosomula of *Schistosoma mansoni*: augmentation by schistosomal binding of eosinophil peroxidase. *American Journal of Tropical Medicine and Hygiene*, **33**, 104–115.
- Jungmann, P., Figueredo-Silva, J. & Dreyer, G. (1991). Bancroftian lymphadenopathy: a histopathologic

- study of fifty-eight cases from northeastern Brazil. *American Journal of Tropical Medicine and Hygiene*, **45**, 325–331.
- Jungmann, P., Figueredo-Silva, J. & Dreyer, G. (1992). Bancroftian lymphangitis in northeastern Brazil: a histopathological study of 17 cases. *Journal of Tropical Medicine and Hygiene*, **95**, 114–118.
- Kar, S. K., Mania, J. & Kar, P. K. (1993). Prevalence of lymphatic nodule in a bancroftian endemic population. *Acta Tropica*, **55**, 53–60.
- Kephart, G. M., Gleich, G. J., Connor, D. H., Gibson, D. W. & Ackerman, S. J. (1984). Deposition of eosinophil granule major basic protein onto microfilariae of *Onchocerca volvulus* in the skin of patients treated with diethylcarbamazine. *Laboratory Investigation*, **50**, 51–61.
- Klei, T. R., Enright, F. M., Blanchard, D. P. & Uhl, S. A. (1981). Specific hypo-responsive granulomatous tissue reactions in *Brugia pahangi*-infected jirds. *Acta Tropica*, **38**, 267–276.
- Klei, T. R., Enright, F. M., Blanchard, D. P. & Uhl, S. A. (1982). Effects of presensitization on the development of lymphatic lesions in *Brugia pahangi*-infected jirds. *American Journal of Tropical Medicine and Hygiene*, **31**, 280–291.
- Klei, T. R., McVay, C. S., Dennis, V. A., Coleman, S. U., Enright, F. M. & Casey, H. W. (1990). *Brugia pahangi*: effects of duration of infection and parasite burden on lymphatic lesion severity, granulomatous hypersensitivity, and immune responses in jirds (*Meriones unguiculatus*). *Experimental Parasitology*, **71**, 393–405.
- Lawrence, R. A. (1996). Lymphatic filariasis: what mice can tell us. *Parasitology Today*, **12**, 267–271.
- Lichtenberg, F. (1957). The early phase of endemic bancroftian filariasis in the male: pathological study. *Mount Sinai Journal and Medicine*, **26**, 983–1000.
- Lichtenberg, F. (1987). Inflammatory responses to filarial connective tissue parasites. *Parasitology*, **94**, S101–S122.
- Lichtenberg, F. & Medina, R. (1957). Bancroftian filariasis in the etiology of funiculoepididymitis, periorchitis and hydrocele in Puerto Rico (Statistical study of surgical and autopsy material over a 13-year period). *American Journal of Tropical Medicine and Hygiene*, **6**, 739–750.
- Mantovani, A., Bussolino, F. & Dejana, E. (1992). Cytokine regulation of endothelial cell function. *FASEB Journal*, **6**, 2591–2599.
- Michael, E., Bundy, D. A. P. & Grenfell, B. T. (1996). Re-assessing the global prevalence and distribution of lymphatic filariasis. *Parasitology*, **112**, 409–428.
- Michael, P. (1945). Filariasis: histopathologic study. *Naval Medical Bulletin*, **45**, 225–236.
- Nelson, F. K., Greiner, D. L., Shultz, L. D. & Rajan, T. V. (1991). The immunodeficient SCID mouse as a model for human lymphatic filariasis. *Journal of Experimental Medicine*, **173**, 659–663.
- Norões, J., Dreyer, G., Santos, A., Mendes, V. G., Medeiros, Z. & Addiss, D. (1997). Assessment of the efficacy of diethylcarbamazine on adult *Wuchereria bancrofti* in vivo. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **91**, 78–81.
- O'Connor, F. W. (1932a). Some pathological changes associated with *Wuchereria bancrofti* infections. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **25**, 445–454.
- O'Connor, F. W. (1932b). The aetiology of the disease syndrome in *Wuchereria bancrofti* infections. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **26**, 13–47.
- O'Connor, F. W. & Hulse, C. R. (1935). Studies in filariasis. I. In Puerto Rico. *Puerto Rico Journal of Public Health and Tropical Medicine*, **11**, 167–367.
- O'Connor, F. W., Golden, R. & Auckinclos, H. (1930). The roentgen demonstration of calcified *Filaria bancrofti* in human tissue. *American Journal of Roentgenology and Radium Therapy*, **23**, 495–502.
- Olszewski, W. L., Jamal, S., Manokaran, G., Lukomska, B. & Kubicka, U. (1993). Skin changes in filarial and non-filarial lymphoedema of the lower extremities. *Tropical Medicine and Parasitology*, **44**, 40–44.
- Ottesen, E. A. (1992). Infection and disease in lymphatic filariasis: An immunological perspective. *Parasitology*, **104**, S71–S79.
- Piessens, W. F., Fuhrman, J. Á. & Vickery, A. C. (1990). Immunology of lymphatic filariasis. In *Modern Parasite Biology*, ed. Wyler D. J. pp. 289–311. New York: W. H. Freeman.
- Racz, P., Tenner-Racz, K., Buttner, D. W. & Albiez, E. J. (1982). Ultrastructural evidence for eosinophil-parasite adherence (EPA) reaction in human onchocercal lymphadenitis in the early period following diethylcarbamazine treatment. *Tropenmedizin und Parasitologie*, **33**, 213–218.
- Rifkin, H. & Thompson, K. J. (1945). Structural changes in early filariasis. *Archives of Pathology*, **40**, 220–224.
- Rothenberg, M. E., Owen, W. F., Silberstein, D. S., Soberman, R. J., Austen, K. F. & Stevens, R. L. (1987). Eosinophils cocultured with endothelial cells have increased survival and functional properties. *Science*, **237**, 645–647.
- Shwartz, D. A. (1997). Emerging and reemerging infections. Progress and challenges in the subspecialty of infectious disease pathology. *Archives of Pathology and Laboratory Medicine*, **121**, 776–784.
- Snowden, K. F. & Hammergerg, B. (1989). The lymphatic pathology of chronic *Brugia pahangi* infection in dogs. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **83**, 670–678.
- Taylor, M. J., Bilo, K., Cross, H. F., Archer, J. P. & Underwood, A. P. (1999). 16S rDNA Phylogeny and ultrastructural characterization of *Wolbachia* intracellular bacteria of the filarial nematodes *Brugia*

- malayi*, *B. pahangi*, and *Wuchereria bancrofti*. *Experimental Parasitology*, **91**, 356–361.
- Vane, J. R., Ånggard, E. E. & Botting, R. G. (1990). Regulatory functions of the vascular endothelium. *New England Journal of Medicine*, **323**, 27–36.
- Vickery, A. C., Vincent, A. L. & Sodeman, W. A. (1983). Effect of immune reconstitution on resistance to *Brugia pahangi* in congenitally athymic nude mice. *Journal of Parasitology*, **69**, 478–485.
- Vickery, A. C., Albertine, K. H., Nayar, J. K. & Kwa, B. H. (1991). Histopathology of *Brugia*-infected nude mice after immune-reconstitution. *Acta Tropica*, **49**, 45–55.
- Vincent, A. L., Vickery, A. C., Lotz, M. J. & Desai, U. (1984). The lymphatic pathology of *Brugia pahangi* in nude (athymic) and thymic mice C3H/HeN. *Journal of Parasitology*, **70**, 48–56.
- Wartman, W. B. (1944). Lesions of the lymphatic system in early filariais. *American Journal of Tropical Medicine*, **24**, 299–313.
- Witte, M. H., Way, D. L., Witte, C. L. & Berna, M. (1997). Lymphangiogenesis: mechanisms, significance and clinical implications. In *Regulation of Angiogenesis*, eds Goldberg, I. D. & Rosen, E. M. pp. 65–112. Basel, Switzerland: Birkhäuser.