IMMUNOLOGIC ASPECTS OF THE PATHOGENESIS OF
HUMAN ONCHOCERCIASIS

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SUMMARY

Onchocerciasis, or river blindness, is a parasitic disease that affects more than 20 million people globally. The induction of pathology is directly related to the presence and destruction of the microfilarial stages (mf) of this filarial nematode. The disease presents clinically with a wide spectrum of dermal and ocular manifestations, the basis of the variation is believed to involve the immune system. The clinical presentations of infected hosts relate to the intensity of the reactions against the parasite. Anti-microfilarial drugs are also thought to somehow involve the immune system in their pharmacological action. In this study we have investigated some of the factors that might contribute to the pathogenesis, with the aim of gaining a better understanding of the role of immune response in these host inflammatory reactions to *Onchocerca volvulus* parasite. In the first study we have highlighted the clinically most severe form of dermal onchocerciasis, known as reactive onchocercal dermatitis (ROD), one that is often ignored and has not been properly identified. This form has special characteristics and important biological information that could greatly assist the general understanding of the disease as a whole. Amongst the three major foci of the disease in the study country, Sudan, the prevalence of ROD was found to be associated with different environmental and epidemiological characteristics; strikingly higher in the hypo-endemic areas. Including ROD cases in the prevalence will upgrade the level of endemicity of a locality, and often bring patients much in need of treatment into mass treatment programs that currently only treat localities with medium to high levels of endemicity. In the following research studies, we tried to address the immunological characteristics of the clinically different onchocerciasis patients. Then we also investigated the role of genetic polymorphism in the gene encoding receptor that links innate and adaptive immunity, namely, FcγRIIa. Patients with either of two major forms of the clinical spectrum-mild and severe dermatopathology were studied by assaying the antigen-driven proliferation of peripheral blood mononuclear cells and the ability of patients’ serum antibodies to promote cytoadherence activity to mf in vitro. Immune responses of those with severe skin disease were found to be stronger compared with the mild dermatopathology group. Mectizan® treatment was followed by an increase in immune responsiveness in those with initially poor responses. Thus the degree of dermatopathology is related to the host’s immune response against mf and immunocompetence may be necessary for Mectizan® to clear the infection efficiently. The infection has also been associated with increased levels of circulating immune complexes (CIC) containing parasite antigens and a cytokine response that involves both pro-and anti-inflammatory cytokines. Our fourth paper investigated the effect of IC from the *O. volvulus* infected patients on the production of pro-and anti-inflammatory cytokines. CIC were increased in all patients studied. The precipitate from plasma treated with polyethylene glycol (PEG) were added to peripheral blood mononuclear cell (PBMC) cultures, and the levels of IL-10, tumor necrosis factor TNF-α, IL-1β and their endogenous antagonists soluble TNF-Rp75 and IL-1-receptor antagonist (IL-1ra) were measured. A significant induction of all cytokines measured occurred in the onchocerciasis patients compared to healthy controls. However, the IL-1ra level was suppressed. The suppression of the production of IL-1ra suggests that the IC containing antigens may have a selectively suppressive effect on the production of this anti-inflammatory cytokine; thus implicating its possible role in counteracting inflammatory responses associated with the disease, and suggesting a potential therapeutic significance. FcγRIIa receptors are involved in many important biological responses, and considered as important mediators of inflammation. A polymorphism in the gene encoding this receptor, that is either arginine (R) or histidine (H) at position 131, affects the binding to the different IgG subclasses. We therefore hypothesized that this polymorphism might be one of the underlying mechanisms to the varied clinical presentations seen in this disease. FcγRIIa genotyping was carried out by gene specific polymerase chain reaction (PCR) and allele-specific restriction enzyme digestion of DNA from clinically characterized patients. The genotype R/R frequencies were found to be significantly higher among patients with the severe form of the disease (including ROD), and it was particularly associated with one tribe (Masaleet) compared to Fulani. Moreover, the H allele was found to be associated with lower risk of developing the severe form. As no significant difference was seen between onchocerciasis cases and controls, the study also implies that this polymorphism influences protection from developing the severe form rather than being related to protection from the infection.
To my dear parents, Mahmoud & Khadija

To my loving wife Suzy, and to Soofi, Noosa, Hammu & Luji
ORIGINAL PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:


(IV) **Magdi M. M. Ali**, Erik Åhlin, Linda Mathsson, Charles D Mackenzie, Suzan I. A. Noori, Klavs Berzins, Gehad ElGhazali and Johan Rönnelid (2005). Stimulation of TNF-α, IL-1β and IL-10 production, but suppression of production of IL-1ra by high molecular weight immune complexes from *Onchocerca volvulus*-infected Sudanese patients: implications for disease pathogenesis and therapy. *(Submitted)*.


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<th>Abbreviation</th>
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<tr>
<td>ADCC</td>
<td>Antibody-dependent cellular cytotoxicity</td>
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<td>AMI</td>
<td>Antibody-mediated immunity</td>
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<td>APC</td>
<td>Antigen-presenting cell</td>
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<td>APOC</td>
<td>African program for onchocerciasis control</td>
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<td>CD</td>
<td>Cluster of differentiation</td>
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<tr>
<td>CIC</td>
<td>Circulating immune complex</td>
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<tr>
<td>Cm</td>
<td>Centimeter</td>
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<tr>
<td>CPM</td>
<td>Count per minute</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>Cytotoxic T-lymphocyte-associated protein-4</td>
</tr>
<tr>
<td>DALY</td>
<td>Disability-adjusted annually lost life years</td>
</tr>
<tr>
<td>DC</td>
<td>Dendritic cell</td>
</tr>
<tr>
<td>DIA-BA</td>
<td>Dot-blot Immunobinding Assay</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked-Immunosorbent assay</td>
</tr>
<tr>
<td>Fc</td>
<td>Fragment crystalizable (contains constant region of the antibody)</td>
</tr>
<tr>
<td>FcγR</td>
<td>Fc gamma receptor</td>
</tr>
<tr>
<td>HFCS</td>
<td>Heat-inactivated fetal calf serum</td>
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<tr>
<td>HSA</td>
<td>Human serum albumen</td>
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<tr>
<td>IC</td>
<td>Immune complex</td>
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<tr>
<td>IFN</td>
<td>Interferon</td>
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<tr>
<td>IL-1</td>
<td>Interleukin</td>
</tr>
<tr>
<td>ITAM</td>
<td>Immunoreceptor tyrosine-based activation motif</td>
</tr>
<tr>
<td>ITIM</td>
<td>Immunoreceptor tyrosine-based inhibitory motif</td>
</tr>
<tr>
<td>Kc</td>
<td>Keratinocyte</td>
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<tr>
<td>Kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>Km</td>
<td>Kilometer</td>
</tr>
<tr>
<td>L3</td>
<td>third stage larval stage</td>
</tr>
<tr>
<td>Mf</td>
<td>Microfilaria/e</td>
</tr>
<tr>
<td>Mm</td>
<td>Millimeter</td>
</tr>
<tr>
<td>NK</td>
<td>Natural Killer</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>OVA</td>
<td><em>Onchocerca volvulus</em> antigen</td>
</tr>
<tr>
<td>PBMC</td>
<td>Peripheral blood mononuclear cells</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffer saline</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>Pers.comm.</td>
<td>Personal communication</td>
</tr>
<tr>
<td>PHA</td>
<td>Phytohaemagglutinin</td>
</tr>
<tr>
<td>RA</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>ROD</td>
<td>Reactive Onchocercal dermatitis</td>
</tr>
<tr>
<td>RPMI</td>
<td>Rosewell Park Memorial Institute</td>
</tr>
<tr>
<td>SLE</td>
<td>Systemic lupus erythematos</td>
</tr>
<tr>
<td>TCR</td>
<td>T-cell receptor</td>
</tr>
<tr>
<td>TGF</td>
<td>Transforming growth factor</td>
</tr>
<tr>
<td>Th1/Th2</td>
<td>T helper1, T helper2</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>Tr</td>
<td>T-regulatory cell</td>
</tr>
<tr>
<td>WHO</td>
<td>World health organization</td>
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INTRODUCTION

General Introduction

Filariae are a distinct group of nematode parasites. All those that infect humans are classified in the family Onchocercidae and the superfamily Filarioidea. The name filaria is derived from the Latin word *filum* meaning thread, and refers to the thread-like morphology of the adult worm. Filariae share common features, including an adult stage that resides outside the digestive tract, the requirement of an obligate hematophagus arthropod vector as an intermediate host, and the release of motile offspring called microfilariae (mf) into the vertebrate host. Filarial worms continue to present a serious challenge to those concerned with public health and to control programs in many parts of the world.

Seven principle species of filariae infect humans, and these are typically grouped into three categories. The lymphatic filariae, consisting of *Wuchereria bancrofti* and *Brugia malayi* or *Brugia timori*, reside as adults in the lymphatics and infect some 120 million persons (Ottesen et al., 1997). Infection with these parasites may cause acute or chronic obstruction of the lymphatics that ultimately leads to elephantiasis. Onchocerciasis is caused by *Onchocerca volvulus*; the adult worms live in subcutaneous nodules or in deeper skeletal tissues associated with major bones. The remaining filariae including *Loa loa*, *Mansonella perstans*, *Mansonella ozzardi*, and *Mansonella streptocerca*, are less important as causes of disease and suffering, although *L. loa* can provoke temporary inflammatory swelling (calabar), hypereosinophilia and other allergic reactions. *L. loa*’s habit of migrating widely throughout the body is especially disturbing if it crosses the eye and surrounding tissues. Moreover, recent reports showed its implications in the development of severe adverse events following ivermectin administration to onchocerciasis patients (Gardon et al., 1997a).

Adult filariae are typically long, slender worms, with pronounced sexual dimorphism; females are characteristically much longer than males. Adult worms are observed rarely
because of their location in the host, so the mf are the usual diagnostic stage. They are generally not infective for non-human vertebrate hosts and undergo no further development in the humans where they survive for 1-2 years.

Onchocerciasis or ‘river blindness’- one of the major filarial diseases of humans- is caused by \textit{O. volvulus}, and transmitted by the female black fly of the genus \textit{Simulium}. It is a leading cause of preventable blindness and a severe pruritic skin condition in endemic areas, and thus one of the most important public health and socio-economic problems faced by the rural populations in these areas (WHO, 1976). More than 120 million individuals are at risk of infection, and over 18 million are presently infected (WHO, 1995). The majority of patients suffer from variable skin lesions. About one million individuals suffer visual impairment as a result of onchocerciasis, with at least 340,000 cases of blindness attributable to the disease. More than 46,000 individuals lose their vision every year as a result of this devastating disease. The impact of blindness on a community is reflected in an increased mortality rate, the mortality amongst blind people being four times higher than that of non-blind persons of the same age in a community (WHO, 1994). The disease is endemic in large areas of Africa, on the Arabian Peninsula, as well as in central and South America. It mainly affects small isolated and more remote communities; a favorable ecology for the intermediate host black fly determines the distribution of the disease. The vector breeds in fast flowing rivers, hence the name “river blindness”. The effect of the disease in communities includes the deterioration of living conditions and desertion of fertile riverside lands by the villagers (WHO, 1987). Fear of the disease has led to the depopulation of river valleys where the vector breeds. It exerts a burden of a million disability-adjusted annually lost life years (DALY) (Brattig, 2004). Therefore, the disease poses a significant obstacle to socioeconomic development. In the past, the stigma of onchocerciasis has always been associated with the blindness caused by this disease, but it is now clear that the onchodermal skin disease has affected both, mental health
and social acceptability leading to a negative impact on the quality of life (Amazigo, 1994; Brieger et al., 1998; Vlassoff et al., 2000). The black fly *Simulium damnosum* is the main vector of *O. volvulus* in Africa (Crosskey, 1969). Several sub-species of *S. damnosum* are distributed worldwide. The male black flies feed only on plant juices and have no role in the transmission of onchocerciasis.

The female flies feed on blood and can take up to 1 mg of blood at each meal. The eggs are laid 3-5 days after the meal. Within 24 hours of laying the eggs, the female fly takes another blood meal. The bite, which involves the creation of a blood pool under the epidermis, is not always noticed immediately, but may be followed by debilitating severe itching. *Simulium* can fly for up to 80 km in 24 hours along the water sources by the aid of their strong thoracic muscles. They may even go hundreds of kilometers with the help of wind from one river basin to another.

**Life Cycle of Onchocerca volvulus**

The life cycle of the parasite in the host still holds some mysteries, due to the lack of a truly suitable comparable animal model and the dearth of postmortem information. The life cycle stages take place in two hosts, the human primary host and the black fly as the intermediate host. As an infected female black fly takes a blood meal from the human, it injects the infective parasitic larvae (known as L3) into the skin. It is proposed that the larvae penetrate the superficial layers of the skin, migrate and develop into adult worms elsewhere in the body over a period of 6-12 months. Within 1-3 years after infection, onchocercal nodules appear in subcutaneous tissues, usually at sites of bony prominences such as the hips and ribs. These nodules are formed around coiled mature worms and are encapsulated by host reactive tissues with a rim of vascularized fibrous tissue; they usually contain both male and female adult worms. The adult males are 2 –5 cm in length and 0.02 mm in diameter, while the females are 50 –70 cm in length and 0.04-0.06 mm in diameter. Fertilized females give birth to live
embryos, about 500,000 to 1,000,000 per year through out her sexually active life (up to 15 years). It has been estimated that the total number of mf distributed throughout the dermis, the eyes, and other parts of the body, in heavily infected individuals may reach 100-150 million (Duke, 1993). They are not commonly found in blood, but may be detected in the urine (Akogun & Tembo, 1996). The mf may live for 1-1.5 years.

*O. volvulus* develops through a number of stages in the female black fly. On biting an individual with dermal mf, the fly ingests mf, which pass to the stomach, where most of them are digested. However a few mf may survive and then pass to the thoracic muscle, where they moult twice to become infective filariform larvae measuring 650 microns in length. These latter forms migrate to the tip of the proboscis in the mouthparts and are transmitted to a human during a subsequent blood meal. The number of infective larvae in one black fly is usually < 10 (WHO, 1987). The cycle of development in the black fly takes about 7 days at 27 - 30 °C, but can last up to 10 or 12 days at lower temperatures. Development stops when temperature falls below 16° C, as often happens at night (Duke, 1968). *O. volvulus* can also be pathogenic to the black fly: on biting an individual with very heavy loads of mf, the fly may ingest several hundreds mf, which can destroy the fly’s own tissues during migration and development, thus preventing flight and other functions (WHO, 1987).
Figure 1 Life cycle of the parasite

Source: (WHO, 1995).
CLINICAL MANIFESTATIONS

The pathogenesis of filarial diseases is characterized by acute and chronic inflammation. The parasite, the immune responses, and/or consequent opportunistic infections initiate these inflammatory responses leading to overt manifestations.

The clinical changes seen in human onchocerciasis are believed to be associated with the destruction of the mf stage of *O. volvulus* in skin and eyes. Clinical disease is caused by the mf stages of *O. volvulus*, with inflammation and tissue damage mostly seen when the parasite is dying or being destroyed by drug and/or host responses. A papular eruption in the skin leads to varying degrees of dermal and epidermal pathology, which is complicated by self-inflicted trauma due to the intense pruritic nature of the infection. Thus, the consequent pathology represents the cumulative tissue and functional outcomes of a long-standing interplay between host and parasite, often lasting many decades. Severe skin changes take place, making it one of the most devastating infectious diseases of human skin. However, the disease is best known for its ocular pathology, which can affect many parts of the eye, sometimes ultimately leading to an irreversible damage and blindness. Noteworthy, a potential factor that could play a role in the development of pathology and clinical symptoms in this disease is the obligatory endosymbiotic bacterium *Wolbachia*, which has been rediscovered and found to be abundant in all developmental stages of the parasite (Bandi *et al.*, 2001; Saint Andre *et al.*, 2002; Taylor *et al.*, 2005).

The adult male and female worms are encapsulated in subcutaneous nodules, usually palpable on bony prominences such as iliac crest, hip, ribs knees and skull. The period between infection with infective larvae and the production of mf by fertilized adult worms (the prepatent period) varies from 7 months to more than 2 years. Generally, clinical symptoms are not present during this period. However, the immature worms may stimulate some immunological responses (Duke, 1991). The time from invasion by infective larvae to
the development of clinical signs (the incubation period) is usually longer than the prepatent period and may last for many years; it is much longer for the manifestation of the ocular disease than the dermal presentations (WHO, 1987).

**Dermal Onchocerciasis**

The skin is the principal site of infection, a broad spectrum of skin manifestations can result from inflammatory reactions associated with damage due to the disintegration of the mf. During the early skin lesions, mf are found primarily in the dermis, and cause no observable reaction as long as they are alive. It is only after the death of mf that inflammatory responses appear. However, it is debatable as to which comes first, parasite death or the host’s responses. Circulating antigens released from disintegrating mf may be deposited into blood vessels and connective tissue in the skin and result in pathology. Eosinophils, neutrophils and macrophages aggregate around dead or dying mf. The clinical manifestations vary according to the intensity of infection (mf load in the skin), the immune responses of the human host, the duration of the infection, and also to inherited factors (Mackenzie *et al.*, 1987; Murdoch, 1992; Soboslay *et al.*, 1997). The commonest early symptoms of the disease include irritation of the skin, pruritus that can be accompanied by oedema and a sensation of hypothermia. Murdoch *et al.* systematically classified cutaneous changes into acute papular, chronic popular and lichenified or reactive onchodermatitis, atrophy and depigmentation, and also graded them (Murdoch *et al.*, 1993). The chronic infection may present itself as depigmentation (leopard skin), a striking feature seen mostly in the shins. These lesions are white and interrupted by foci of persistent pigmentation around the pores and hair follicles, it is more often observed in older infected people. In later stages, the skin loses elasticity and may become wrinkled and hang in loose folds (hanging groin) (Salih, 1985); this is often associated with local underlying lymphadenopathy (Mackenzie, pers.comm.).
A majority of the infected people develop a generalized form of the disease, characterized mostly by a high live mf load with mild or minimal degree of inflammatory processes in the skin. This is in contrast to a small group of patients with localized form of the disease who have low, if any, dermal live mf and only few nodules with adult worms. The latter condition or syndrome known as chronic hyper-reactive onchodermatitis or sowda (the Arabic word for black), was first described in Yemen (Fawdry, 1957) and then in West Africa (Bartlett et al., 1978), and in eastern Sudan (Ghalib et al., 1987). The affected area of skin is intensely pruritic, dark and thickened. The condition is usually localized and typically involves only one lower extremity. The live mf load is extremely low and even sometimes undetectable. However, the impact of pruritus is definitely reflected on the general health appearance of the infected individuals, leading to noticeable weight loss in people in the hyperendemic areas (Burnham, 1991).

**Nodules (Onchocercomata)**

Many patients develop fibrous subcutaneous masses containing the adult onchocercal worms know as (nodules). They are painless in themselves (unless pressing on a vital organ), mobile and 0.5–1.0 cm in diameter. They can group together to form conglomerates as big as 6-8 cm in diameter containing 10-15 individual worm masses. They are mostly seen over bony prominences of the trunk, thighs, shoulders, arms and cranium (Krupp & Chatton, 1978). However, some nodules lie deeper and are impalpable. The sites of the nodules depend in part on the biting habits of the black fly vector (Duke, 1972), for example in South America it is mostly seen on the skull.

Infection of the breast was found to rarely be involved in onchocerciasis, and could lead to calcification of the breast. More recently, a presence of a mass was reported in a breast of infected females, and had a microscopic evaluation consistent with *O. volvulus* (Arribas et al., 2005; Zavieh et al., 2004).
The formation of a nodule seems as a response of the host to an organism continuously producing foreign proteins, causing perivascular infiltration of leukocytes and tissue cells, including fibroblasts and histocytes, thus forming a granuloma (Brattig, 2004). The character of the infiltrate varies in terms of the presence or absence of eosinophils, but macrophages are almost always the predominant cell type. Lymphocytes are most abundant at the periphery of the nodule and surrounding the inner core of a dense inflammatory cell infiltration, composed primarily of macrophages around the adult worm itself. Interestingly, macrophages seem to be the cell type most intimately involved with adult parasites, while the eosinophils are the cell type most prominently interacting with mf. Recently, strong neutrophil infiltrates adjacent to the live adult worms were seen in untreated onchocerciasis patients, unlike those who received doxycycline treatment, and showed drastically reduced accumulation (Hoerauf et al., 2003). This finding may relate neutrophils chemotaxis and activation to the endobacterial products of *Wolbachia* (Brattig et al., 2001; Taylor, 2003). T cells are distributed in a manner suggesting that they play more of an overseer role than one of an effector cell population that is directly anti-parasitic.

**Lymph nodes in onchocerciasis**

*O. volvulus* affects lymph nodes of infected individuals, resulting in a range of inflammatory responses. The acute responses are mast cell-based reactions to cuticular collagen (Gonzalez-Munoz et al., 1999). At chronic stages of this disease, fibrotic and degenerative reactions are seen, and there is a possibility of involvement of autoimmune responses (Eggleton & Llewellyn, 1999; Gallin et al., 1995). Two general forms of presentation exist. The first is seen in the most active, or reactive onchodermatitis “ROD”, in which the lymph nodes are greatly enlarged with follicular hyperplasia, activation of germinal centers, and sinus histiocytosis with varying numbers of plasma cells, eosinophils, and neutrophils. The nodes are firm but not painful or tender; the inguinal and femoral nodes being the most commonly
affected. Histologically, these lymph nodes are active in the cellular and humoral arms of the immune response. The second general form is as shrunken, hard feeling and fibrous lymph node or group of lymph nodes, and is common in older patients with a long history of infection and chronic degenerative dermal disease (atrophy, depigmentation, etc). Histologically, these lymph nodes are replaced by sclerosing changes and are immunologically quiescent (Mackenzie, pers. comm).

**Ocular onchocerciasis**

The development of ocular lesions correlates with the degree and duration of infection (Fuglsang & Anderson, 1977). In patients with palpable nodules, serious pathology of the posterior segment of the eye was found twice as frequently as in onchocerciasis patients without nodules (Berghout, 1987). Both the anterior and the posterior chambers of the eyes are likely to be affected by the parasite, resulting in diminution of visual acuity or complete loss of vision. Corneal inflammation (keratitis) is a major cause of visual impairment in *O. volvulus* infection. Two distinct forms of corneal diseases are recognized: reversible punctate keratitis (snowflakes opacities) and the irreversible more severe sclerosing keratitis, which has a permanent effect (WHO, 1987). There are geographical differences in the prevalence of onchocercal blindness, being more common in the Savannah areas of West Africa (2-15% of the population of hyper-endemic areas) than in the rain forest areas of West Africa (blindness < 2%). There is evidence for genetic variations amongst *O. volvulus* (i.e. parasite variants) associated with variations in virulence, i.e. in the ability to experimentally infect rabbits (WHO, 1987). Furthermore, when using DNA classification techniques including quantitative polymerase chain reaction (PCR) assay, it was found that there is a strong correlation between disease severity and strain of parasite and also with the relative burden of its endosymbionts (*Wolbachia*) i.e. pathogenicity is strain related and is a function of the relative *wolbachia*
burden (Higazi et al., 2005; Zimmerman et al., 1992). Host genetic factors may also be responsible for this variation (Hoerauf et al., 2002; Murdoch et al., 1997).

It has been postulated that host immunological responses could account for ocular pathology (Bryceson, 1976). Recently, evidence has become available that antigen-specific T cell and antibody responses are essential for the development of O. volvulus keratitis, and the sequence of molecular and cellular events leading to migration of inflammatory cells to the cornea, thus leading to the loss of corneal clarity (Pearlman & Hall, 2000). Also, new findings indicate that neutrophil activation by Wolbachia organisms contribute to the pathogenesis of ocular onchocerciasis (Gillette-Ferguson et al., 2004). Involvement of autoimmunity was suggested, due to the fact that patients continue to show chronic, low-level, progressive pathologic changes of the retina and retinal pigment epithelium, even after chemotherapy to reduce parasite load. In addition, progression of the disease of the retina and optic nerve, unlike that of the cornea, does not appear to be related to mf worm burden (Semba et al., 1990). Molecular mimicry or immunologic cross-reactivity between host and bacterial or viral antigens has been suggested to have a role in the development of a number of autoimmune diseases and has also been suggested to play a role in the development of ocular onchocerciasis. Also, many molecules of the parasite were found to be counteracting the host immune responses, probably to evade destructive mechanisms (Harnett et al., 1999; Pastrana et al., 1998). Other human factors, such as ecological, nutritional, and multiple infections with other parasites, may also influence the clinical appearance of onchocerciasis in different geographical areas.

**IMMUNOLOGY AND IMMUNOPATHOLOGY**

**Immunity to Onchocerciasis**

Onchocerciasis patients are in a delicate immunological balance with their parasite, and the pathologic manifestations of O. volvulus infections result not only from the parasite, but also
from the magnitude and quality of the host immune responses (Ali et al., 2003; Mackenzie et al., 1987). The existence of protective immunity to helminth infections in humans is a controversy. A major impediment to research in onchocerciasis has been the lack of a suitable animal model, and only limited success has been achieved in this context (Abraham et al., 2002; Nanduri & Kazura, 1989; Soboslay et al., 1991). The protective immunity to *O. volvulus* infection in humans is inferred from the presence of a small number of uninfected individuals in hyper-endemic areas. Immunological characterization of such individuals has been pursued, by e.g. determining antigens that might elicit antibody responses responsible for this protection (Elson et al., 1994; Nutman et al., 1991; Steel et al., 1991). Integrating clinical and epidemiological information with PCR and ELISA data, the immune response in putatively immune individuals was found to correlate with low titers of specific IgG and IgE compared to infected individuals (Elson et al., 1994; Ottesen, 1995). Nevertheless, the putatively immune individuals were found to produce significantly more IFN gamma in response to *O. volvulus* antigens and less IL-10 spontaneously than did those in the infected group (Elson et al., 1995; Ottesen, 1995). Thus, protective immunity to onchocerciasis may be mediated in part by an antigen-specific Th1 type response. However, some other reports relate the immunity to the production of IL-5 and the resulting increased eosinophilia (Brattig et al., 2002; Hogarth et al., 1998).

**Cytokines and Onchocerciasis**

T helper type 1 (Th1) and type 2 (Th2) cells represent terminally differentiated effector cells characterized by different cytokine production and homing capacity. The generation of either type of response can confer protection against pathogens or lead to immunopathology. Th1 and Th2 polarization is a stochastic process, which is promoted by interleukin 12 (IL-12) and IL-4 (Trinchieri, 1995). Other factors that contribute to the Th1/Th2 balance are the dose of antigen, strength of antigenic stimulation, duration of T cell receptor (TCR) engagement and
the nature of co-stimulatory molecules (Bird et al., 1998; Gett & Hodgkin, 1998). Immune balance, controlled by Th1 and Th2 cells, is critical for the protection of the host from pathogenic invasion, while its imbalance becomes the cause of various immune disorders. IL-4 and IL-10 regulate antibody production and can suppress cell-mediated immune responses. Distinct type 1 and type 2 T helper cytokines cross-regulate the expression and magnitude of *O. volvulus*-specific cellular responses in humans (Soboslay et al., 1999). However, Doetze (Doetze et al., 2000) and Satoguina (Satoguina et al., 2002) suggested that the antigen-specific cellular hyporesponsiveness in a chronic human helminth infection is mediated by T helper 3/ T helper 1 –type cytokines, but not by a Th1 to Th2 shift. Nevertheless, the Th1 pattern of cytokine production has long been associated with immunity or resistance to helminth infection. A Th2 or type 2-cytokine pattern has been associated with the progressive forms of *O. volvulus* infection in humans (Johnson et al., 1998; Modlin & Nutman, 1993; Soboslay et al., 1999; Timmann et al., 2003; Turaga et al., 2000). However, up-regulation of the pro-inflammatory cytokines during the early human immune response to the infective-stage larvae (L3) suggests that the primary immune response to the live infective stage of the parasite is not predominantly Th2 in nature, but rather dominated by a Th1 response (Babu & Nutman, 2003).

**Natural killer cells (NKs)**

Natural killer (NK) cells are viewed as an important component of innate resistance against a variety of pathogens. NK cells secrete IFN-gamma and have the capacity to activate macrophages before the induction of the antigen-specific T-cell response. Therefore, NK cells might play a role in the balance of Th1 versus Th2 (Bancroft, 1993; Biron et al., 1999). More recently described on NK cells, are the Toll-like receptors (TLRs). Particularly, such receptors of the innate immune system could allow NK cells to directly sense pathogen, and their ligation on accessory cells indirectly activate NK cells through cytokine production.
Interestingly, the first evidence for TLR function in NK cells came from models of parasitic infections. Products from *Leishmania major* stimulated NK cells to produce the pro-inflammatory cytokines IFN-c and TNF-a through cell surface ligation of TLR2 and TLR4 (O'Connor *et al.*, 2005). Noteworthy, it was recently demonstrated that products from *O. volvulus* and its symbionts *Wolbachia* could stimulate pro-inflammatory consequences in a TLR-dependent fashion (Brattig *et al.*, 2004).

**Eosinophils & IgE**

Eosinophilia and elevated serum IgE are immunological hallmarks of infection with parasitic helminths. Immediate hypersensitivity reactions are characterized by the presence of IgE antibodies, eosinophils, mast cells and basophils, all of which have been implicated in resistance to infection as well as pathogenesis. Eosinophils are usually attracted to infection sites leading to macrophage activation and mf killing (David, 1982; Klion & Nutman, 2004). They are poor phagocytes; instead, they take part in defense reactions by secretion of some toxic products, e.g. the eosinophil cationic protein (ECP). Other potentially toxic inflammatory mediators that are released by activated eosinophil include leukotrienes (Shaw *et al.*, 1985), platelet-activating factor (Cromwell *et al.*, 1990) and reactive oxygen species (McCormick *et al.*, 1996).

The level of circulating IgE does not necessarily give complete information about the biological function of this immunoglobulin class, and information gained from specific tests, such as measuring the release of mediators from basophils and mast cells, is usually more useful (Satti *et al.*, 2004; Satti *et al.*, 1996). Using histamine release measurement, it was found that sensitized basophils and immediate hypersensitivity reactions were suggested to play a role in the pathogenesis of onchocerciasis (Satti, pers. comm.).

The clinical changes seen in human onchocerciasis are believed to be associated with the destruction of the mf stage of *O. volvulus* in skin and eyes (Mackenzie *et al.*, 1985;
Piessen & Mackenzie, 1982). The interaction between the immune system and *O. volvulus* that on one hand allows the survival of the parasites for long periods of time, on the other hand promotes the death and removal of the parasite. The diversity in clinical presentation of onchocerciasis is considered to reflect the intensity and quality of immune responses to the parasite or its products (Mackenzie *et al*., 1985). The destruction of mf was reported to involve eosinophils acting through antibody binding leading to low number of macro-and microfilariae (Brattig *et al*., 1991; Green, 1980; Mackenzie *et al*., 1980), and is related to papular eruption *in vivo* and to punctate keratitis lesions in the eye (Mackenzie *et al*., 1985). Interestingly, it has been suggested that there might be significant differences in the susceptibility to and mechanisms of eosinophil-mediated killing between different life-cycle stages of *O. volvulus* parasite (Brattig *et al*., 1991). The antibodies involved in this killing phenomenon were thought to be of the IgG isotype (Brattig *et al*., 1994; Ghalib *et al*., 1985; Ngu *et al*., 1989). Patients with low skin microfilarial loads have high IgG3 and low IgG4 antibody titers, suggesting a protective role for IgG3 and a suppressive one for IgG4 (Dafa'alla *et al*., 1992). Moreover, serum concentrations of IgE and IgG are significantly elevated in sowda patients (Brattig *et al*., 1994; Ottesen, 1995).

Nevertheless, other mechanisms have been suggested as possible contributors in the pathogenesis of this disease. The demonstration of antibodies to retinal S-antigen (S-Ag) in onchocerciasis patients (Chan *et al*., 1987; McKechnie *et al*., 1993) suggests the possibility of involvement of autoimmunity in blinding disease. Moreover, in the ROD form of the disease, autoantibodies that cross-react with defensins of neutrophils are frequently observed, suggesting the role of antigenic mimicry in the immunopathological consequences seen in this unique group of patients (Gallin *et al*., 1995).

IL-4 and IFN-gamma play crucial roles in the regulation of IgE responses in onchocerciasis patients. IL-4 is associated with higher levels of IgE production, while IFN
gamma has been found to down-regulate the IgE. The amount of IgE produced depends on the relative quantity of IL-4 and IFN gamma secreted by parasite-stimulated T cells (King et al., 2001; King et al., 1990; Klion & Nutman, 2004). Furthermore, the induction of IgE by filarial antigens depends on the concentration of the antigens. The mechanism responsible for the induction of IgE by IL-4 appears to involve the regulation of isotype switching to IgE in uncommitted B cells (Bergstedt-Lindqvist et al., 1988). Eosinophilia has also shown to be regulated by T cells, and human IL-5 is a potent stimulus for eosinophil proliferation in vitro (Bergstedt-Lindqvist et al., 1988). In vivo eosinophilia was found to be preceded by increased production of IL-5 following microfilaricidal therapy (Limaye et al., 1993) and Ali et al., unpublished). Interestingly, a recent study in humans demonstrated a quantitative correlation between the in vitro activity of PBMC to produce IL-5 and in vivo effect as reflected by eosinophilia (Brattig et al., 2005).

**Parasite-specific immunosuppression**

Defective responsiveness of peripheral blood lymphocytes in vitro from onchocerciasis patients has been reported and related to duration and intensity of infection (Elkhalifa et al., 1991; Gallin et al., 1988; Green, 1980; Green et al., 1983; King et al., 2001; Soboslay et al., 1991). The balance between the host and the parasite maintained by parasite-derived immunosuppression and other mechanisms is central to the persistence of the infection. The mechanisms underlying immunosuppression are yet to be clearly identified. However, this suppression may be mediated by antigen specific regulatory T-cells (Tr1/Th3) that produce the anti-inflammatory cytokines (IL-10 and transforming growth factor-β) (Satoguina et al., 2002). Recently, it was demonstrated that the length of exposure, mf status and in utero contact with parasite antigen, affect the level of the T-cell co-stimulatory molecule (CTLA-4), leading to a change in the Th1/Th2 balance, thus affecting T-cell responsiveness with time (Steel & Nutman, 2003). Moreover, the parasite’s life cycle multiple stages interact with
different types of antigen-presenting cells (APCs) that might in turn; shape the subsequent adaptive immune responses (Semnani et al., 2004). The major conclusion from these studies is that responses to parasite antigen in patients with the generalized form of onchocerciasis are minimal, compared to putatively immune persons living in endemic areas without active infection and also to those with the severe form including ROD or sowda patients.

Flow cytometric determination of lymphocyte subset distributions show improvement in the CD4⁺ T-cells status of patients following treatment to reduce mf with the microfilaricidal ivermectin. Production of IL-2 and IL-4 by lymphocytes induced by phytohemagglutinin (PHA) increased one month after treatment (Freedman et al., 1991). Treatment with ivermectin seems to enhance cellular proliferative responses, 3-6 months post-treatment (Soboslay et al., 1997; Steel et al., 1991). However, a generalized suppression in lymphocyte proliferation has been observed 2 weeks after treatment (Nutman et al., 1987), probably resulting from the release of suppressive mf antigens (Lal et al., 1990). Several findings support the hypothesis that mf-derived products are important in suppressing lymphocyte proliferative responses to O.volvulus antigens (Elkhalifa et al., 1991; Haffner et al., 1998; Wanni et al., 1997; Williams et al., 1987). Treatment with filaricidal drugs may disturb this balance (Mackenzie et al., 2003). Furthermore, ivermectin has immunostimulatory properties that are associated with altered functions of T-lymphocytes, particularly T-helper cells (Blakley & Rousseaux, 1991). The involvement of the immune system in the anti-filarial efficacy of ivermectin has been raised repeatedly (Ali et al., 2002; Soboslay et al., 1994).

The endosymbiotic bacterium Wolbachia is now believed to play a vital role in the recruitment of the neutrophils implicated in the development of pathology in onchocerciasis patients (Bandi et al., 2001; Brattig, 2004). Moreover, monocytes/macrophages were found to be activated and produce inflammatory cytokines after incubation with extracts from
endobacteria-containing filariae (Taylor et al., 2000). Inflammatory reactions associated with mf destruction and removal constitutes cytokine release and immune complexes (IC) formation and various physical events that often result in tissue damage, however, their pathological significance remains to be clarified. Previous work in Southern Sudan demonstrated the association of higher levels of circulating IC with the disease severity (Sisley et al., 1987). Noteworthy however, host genetic factors also emerged to be important players of determining the intensity of O. volvulus infection and expression of reactive dermatopathology in general (Hoerauf et al., 2002; Murdoch et al., 1997).

**Fc Receptors**

Fc receptors belong to the family of immuno-receptors, which includes T-cell receptors, B-cell receptors and natural killer (NK) receptors. These glycoproteins are expressed on the surface of hematopoetic cells (Ravetch & Bolland, 2001). They are essential molecules in the host defense against infection, providing a link between the cellular and humoral arms of the immune system, allowing for triggering effector responses from cells such as macrophages (phagocytosis), NK cells (antibody-dependent cellular cytotoxicity, ADCC), neutrophils (activation), and B cells (antigen presentation) (Flesch & Neppert, 2000). Fc receptor for IgG-FcγR- display three biochemically and structurally distinct classes of FcγR in humans: FcγRI (CD64), a high affinity receptor that binds monomeric IgG1/3/4, FcγRII (CD32) and FcγRIII (CD16), which are of lower affinity and interact only with complexed or aggregated forms of IgG (Nakamura et al., 2005; Ravetch & Bolland, 2001). Members of the FcγRII class differed from those of other FcγR classes in that they comprise either activitory (ITAM) or inhibitory (ITIM) signaling motifs within their respective ligand-binding chains (Nakamura et al., 2005). FcγRIIa is the most widely distributed FcγR isoform, being expressed on the surface of virtually all myeloid cells, including mononuclear phagocytes, neutrophils and platelets. This receptor plays critical roles in the removal of immune complexes, activation of inflammatory
cells and phagocytosis of antibody-coated microorganisms (Ravetch & Bolland, 2001). Notably, the major receptor for C-reactive protein (CRP) on monocytic cells is FcγRIIa (Bharadwaj et al., 1999). Indeed genetic variation in FcγRs constitutes an important determinant for the host defense capabilities (van Sorge et al., 2003).

**FcγRIIA polymorphism**

The gene encoding FcγRIIA displays a functionally relevant G/A single nucleotide polymorphism in the region encoding its ligand-binding domain, which causes an arginine (R) to be replaced with histidine (H) at position 131 of its extracellular domain (van Sorge et al., 2003). Both allotypes avidly bind complexed human IgG3 and IgG1, but the FcγRIIA-H131 allotype displays a higher affinity for human IgG2 than the FcγRIIA-R131 allotype; none of them bind IgG4 efficiently. Because FcγRIIA-H131 is the only FcγR which interacts efficiently with human IgG2, this allotype is essential for the clearance of IgG2-containing immune complexes (Salmon et al., 1996) and phagocytosis of IgG2-opsonised microorganisms (Sanders et al., 1995). As a consequence, the FcγRIIA-R/H131 polymorphism is associated with predisposition to autoimmune diseases, which may be mediated by the deposition of IgG2-containing immune complexes (Karassa et al., 2004), and infections caused by encapsulated bacteria, whose clearance largely depends on IgG2-mediated phagocytosis (Jansen et al., 1999; Rodriguez et al., 1999). Thus, host defense against pathogens including *O. volvulus* likely depend on cellular activities that could in turn be influenced by the FcγRIIa polymorphism. Investigation the relationship between this polymorphism and disease outcome could contribute to better understanding the underlying mechanisms of onchocerciasis in different ethnic groups. Of note, however, reports addressing the polymorphism and onchocercal clinical manifestations are currently lacking in the literature. Indeed genetic variations and polymorphisms in FcγRs, TLRs (proposed to be
crucial in immune responses against *Wolbachia*). ECP (Eosinophils are thought to play crucial role in mf destruction); and some other gene candidates constitute important determinants for host defense capabilities. Further work is needed to better understand the long standing interplay between infection status, the balance between immune responsiveness and immune modulation.

**DIAGNOSIS**
Filariasi in general continues to present diagnostic challenges. The broad spectrum of clinical manifestations associated with this infection creates a diagnostic paradox. The accurate and specific diagnosis of filarial infection has become increasingly important as a mean of monitoring the efficacy of mass distribution of Mectizan®. This goal has been hampered by our inability to distinguish between past and current infection.

**Parasitological diagnosis of infection**
The detection of unsheathed mf in the skin involves removing small snips of skin and incubating them in an aqueous solution, preferably RPMI 1640 to allow mf to migrate out, so that they can be observed microscopically. A bloodless skin snip weighing from one to several micrograms can be removed quickly and without much discomfort, is obtained using a razor blade, or Walser- type corneoscleral punch. Mf will emerge from the sample within 4 hrs, and the intensity of infection is reflected in the number of mf emerged from the snip (Taylor *et al.*, 1989). Sowda or ROD patients have a low load of viable mf, or may even show a negative test by this method. Skin from the pelvic girdle provides the best chances for detection (Williams *et al.*, 1985a; Williams *et al.*, 1985b).

**Ultrasonography**
Onchocerciasis can also be diagnosed by detecting mf in the eye by using the slit-lamp. Detection of subcutaneous nodules is also used for clinical diagnosis, and the introduction of ultrasound facilitated the detection of these nodules (Homeida *et al.*, 1986; Leichsenring *et
Onchocercal nodules must always however, be distinguished from other similarly presenting lesions such as lipomas, foreign bodies, granulomas, and sebaceous and dermoid cysts. Ultrasonographical examinations of onchocercomas, where living adult filariae can be displayed, may well serve as a new tool for the longitudinal observation in vivo of patients with onchocerciasis undergoing treatment and as an adjunct to histological evaluation (Mand et al., 2005).

**Immunological and Molecular Diagnosis:**

The Mazzotti test - (after the Mexican Luis Mazzotti, who first described them) - is an allergy-based reaction, was frequently used as a diagnostic aid for patients with negative skin snips. It is carried out by administration of Diethylcarbamazine (DEC) 0.5 mg orally every 4-6 hours for three days and then 1 mg every 4-5 hours. Dermal changes were seen, consisting mainly of papular responses (El Sheikh et al., 1986; Stingl et al., 1984). However, more recently the skin patch test is used, and proved to be more sensitive than skin snipping, non-invasive, simple and cheap. It lends itself as a more relevant tool for surveillance in low prevalence area (Boatin et al., 2002).

Also, new procedures have been developed for the immunodiagnosis of onchocerciasis, and these will be of great significance, since the detection of mf by using skin snip is not feasible in prepatent infections or in those with strong immunological responses. Circulating antibodies are detectable using an ELISA system with blood obtained from finger prick and collected onto filter paper. This test is useful in those under 15 years and could be integrated with other disease programs that monitor blood samples, like malaria (Botto et al., 1999). Recombinant *O. volvulus* antigen candidate (Ov20/OvS1) was demonstrated to aid in the diagnosis of onchocerciasis and found to be useful candidate antigen particularly for the detection of patients with reactive form of the disease, ROD or sowda (Mpagi et al., 2000).
More recently, dot blot immunobinding assay (DIA-BA) based on the biotin-avidin binding system, for the detection of *O. volvulus* specific antigens in body fluids was suggested (Wembe *et al.*, 2005). The use of other species of *Onchocerca* parasite as sources of antigens as diagnostic tools has also been reported (Cho-Ngwa *et al.*, 2003).

A PCR test for skin snip samples, to detect the presence of *O. volvulus* DNA in order to rapidly assess the epidemiology of the disease in endemic areas (Zimmerman *et al.*, 1994), was also found to be more sensitive than standard skin snip examination.

**Epidemiological Diagnosis**

Diagnostic procedures in onchocerciasis are used to determine the prevalence of infection, to identify individuals requiring treatment, to evaluate the success of treatment, and to assess the impact of control efforts. A non-invasive epidemiological technique is needed for the detection of disease to determine eligibility of mass treatment programs, as has been launched by the WHO in the African Program for Onchocerciasis Control (APOC). This technique is known as Rapid Epidemiological mapping for Onchocerciasis (REMO). It comprises a rapid search for nodules in individuals suspected to have the infection. It requires experience and knowledge for the personnel who perform the technique. It greatly helped to estimate endemicity in order to start the control program (Noma *et al.*, 2002).

**DISEASE CONTROL**

**Treatment**

Treatment of onchocerciasis has been a problem in the past and is not yet satisfactory. Ideal treatment of infection with *O. volvulus* would include drugs that kill the adult worm (macrofilaricidal) and the mf (microfilaricidal), with minimal side effects on those who receive this treatment. The history of treatment involves suramin, DEC and recently ivermectin (Mectizan®) which is currently the drug of choice. Suramin was administered intra-venously in repeated doses, as a macro- and micro-filaricidal, but the drug had severe
cumulative toxicity effects because of its very slow excretion. DEC was administered orally in a multiple dose regimen(s), but was associated with a very high increase of adverse reactions, resulting from the rapid killing of mf in the skin, subcutaneous tissues and the eyes. This can lead to life threatening severe allergic-like clinical responses, including severe itching, lacrimation, urticarial oedema of the skin, swelling and tenderness of the lymph nodes, maculopapular eruption, pyrexia and sometimes severe symptomatic postural hypotension (Awadzi, 1980; Awadzi, 2003; Elkhalaifa et al., 1985). In patients with ocular lesions, DEC aggravates the condition and can cause complete loss of vision (Bird et al., 1980). Thus, patients treated with Suramin or DEC should be hospitalized or treated under close medical supervision.

In view of these problems there was a desperate need for an alternative therapy for onchocerciasis. Ivermectin is a semi-synthetic macrocyclic lactone produced by the actinomycete Streptomyces avermitilis sp. developed by Merck & CO., Inc. It has been extensively used in veterinary medicine for treating internal and external parasites (Campbell, 1985). The drug was introduced for human onchocerciasis (WHO, 1987). It is considered one of the milestones of tropical disease treatment and a revolutionary breakthrough, as it has an effective microfilaricidal action that could clear microfilariae from the skin with minimum side effects (Awadzi, 1980; Aziz et al., 1982; Baraka et al., 1995b; Campbell, 1991). The drug is given in a single dose and produces a prolonged reduction in microfilarial loads. It has provided for the first time a feasible chemotherapy for large-scale treatments. It has been used successfully in mass treatment programs (Baraka et al., 1995a). It improves skin lesions except for depigmentation (Amazigo et al., 2004; Pacque et al., 1991). Recently, a model for human onchocerciasis (O. ochengi infection in cattle) demonstrated the effectiveness of administering the antibiotic tetracycline together with ivermectin, resulting in a macrofilaricidal effect. This response is hypothesized to be related to the action of tetracycline.
on *Wolbachia endobacteria*; abundant in *O. ochengi* (Trees et al., 2000). This combination could be a useful procedure that may increase options for the control of human onchocerciasis. Recent findings support a role for *Wolbachia* products in mediating the inflammatory responses seen following treatment of onchocerciasis and suggest new targets for modulating these reactions (Keiser et al., 2002).

Ivermectin uptake was thought to be mediated by specific high affinity receptor. It is suggested to have a significant effect in the release of mf from the female gravid uterus by reducing the number of the multicellular embryogenic stages in worms exposed to multiple doses of ivermectin. This may be partially due to the reduction in the effectiveness of insemination in female worms and minor impairment of oogenesis (Chavasse et al., 1993). However, recently some recurrence was observed in a small proportion of treated patients with ivermectin after only one month of treatment, unlike previous reports. This also raises the possible involvement of the immune responses in the microfilaricidal mechanism of action of ivermectin. Studies concerning immunological responses after repeated doses of ivermectin in patients with onchocerciasis emphasized the apparent long term safety of ivermectin, through the demonstration of the absence of immunopathological responses induced by repeated ivermectin treatment (Steel et al., 1991). The immunosuppression due to the disease is reversible after ivermectin treatment and thus ivermectin may be needed to enhance immunity against onchocerciasis (Soboslay et al., 1994). It might also be involved in the mechanism of killing by the drug.

APOC-WHO adopted a control strategy for combating onchocerciasis, which relies mainly upon ivermectin treatment. This new approach employs a community directed treatment strategy to secure the issue of sustainability. As the drug will only kill the microfilarial stage, treatment should be repeated at least once a year for at least 12-15 years,
the expected life span of the adult worm. The program started in nineteen African countries and has treated more than 12 million individuals.

The present contraindication criteria for ivermectin treatment include pregnancy; age <5 years or wt <15 kg and breast-feeding women up to one week after birth. Sickness in individuals or concurrent illness should also be borne in mind in the treatment campaigns. The involvement of the central nervous system (CNS) in the pathogenesis of the infection needs to be considered especially the life threatening adverse events to ivermectin treatment of patients co-infected with the filarial parasite *Loa loa* (Ducorps et al., 1995; Gardon et al., 1997a; Gardon et al., 1997b). Currently, with the introduction of the Mectizan-Albendazole program for lymphatic filariasis, the mechanisms for handling and minimizing the problems where the two diseases are co-endemic is considered essential part of control program management.

In conclusion, ivermectin alone has been extremely successful so far aiming at eliminating onchocerciasis as a public health problem. However, elimination of transmission has proved to be more difficult. Noteworthy however, the possibility of parasitological unresponsiveness to Mectizan treatment or selection for drug resistance in *O. volvulus* has been reported (Boussinesq & Gardon, 1998). Thus; emphasis on the development of methods for detection of ivermectin resistance has also been considered. Research is going on to identify new drugs that can kill adult worms without harmful side effects, or have long-term sterilizing effects. Current studies are focused on moxidectin as a potential macrofilaricidal. It was recently demonstrated to be safe and well tolerated in humans (Cotreau et al., 2003).

Recent efforts also focused on the use of existing antibiotics as alternative treatments for the elimination of *Wolbachia* which have emerged as a new target for treatment with drugs that lead to long term sterilization of adult female filarias (Hoerauf et al., 2003). Chemotherapy with doxycycline (currently for six weeks) could be used to treat infected individuals. This approach holds promise for new developments based on registered antibiotics that are
affordable in resource poor settings, as extensive registration processes are not needed (Hoerauf et al., 2003). However, the relatively long duration of treatment rules out the possibility to replace ivermectin as a control measure (mass treatment), not to mention the known contraindications (age <9 years, pregnancy).

**Nodulectomy**
Surgical removal of nodules from accessible parts of onchocerciasis patients helps in reducing the parasite burden in the body and hence decreases the severity of the disease. But many nodules are not easily detectable. Therefore, this is not a feasible way of control strategy as it is very cost ineffective, and needs hospital support which is not available in most situations or cases of the remote areas where the disease prevails.

**Vaccine development**
Major steps have been taken toward the control of the disease, however, with all the available tools; onchocerciasis seems to be ineradicable at least in Africa. Thus, an alternative strategy may be to seek a high desirable tool to guarantee sustained elimination success, which could be a vaccine. However, the prerequisite for vaccine development is an improved understanding of the mechanisms that govern protective immunity to onchocerciasis in humans. As stated earlier one significant obstacle in studies of the immune responses to *O. volvulus* has been the limited host range. Another handicap is the difficulty to obtain live fresh larval stages.

Alternative strategies were used to isolate potentially protective antigens include targeting molecules that are considered to be critical to the infection process. Complementary DNA (cDNA) libraries have been prepared for the adult and larval forms of the parasite to serve as a source of recombinant antigens for use in vaccine trials (Lustigman et al., 2002). The presence of putatively immune individuals suggests that protective immunity to filarial parasite may occur naturally.
THE PRESENT STUDY

Rationale

*Onchocerca* species induce both cellular and humoral immune reactions. The intensity of these reactions varies considerably with the clinical manifestations of the infected hosts. Infections are chronic, and persistence of the parasites for several years argues for highly adapted mechanisms of immune evasion. Cellular responses to parasite antigens are suppressed in individuals with high parasitemia “generalized onchocerciasis”, unlike in patients either free from infection, or those manifesting localized disease with very low live mf loads if any. The mechanisms that control the levels of circulating mf *in vivo* are not well understood. Speculations to the process of *in vivo* killing of the mf have been made. Onchocerciasis persists in most of the treated patients who continue to live in endemic areas. A degree of resistance to the infection may exist. Many questions in onchocerciasis concerning the interplay between infection status and the balance between the immune responsiveness and immune modulation remain to be addressed. The propensity of some patients to develop the extremely debilitating condition known as ROD has been a matter of controversy. The probable susceptibility could be understood more clearly by defining the interplay between biological and genetic characteristics among those affected to provide insights into both susceptibility and pathogenesis. The disease has also been associated with increased levels of circulating immune complexes (IC) and a cytokine response including both pro-and anti-inflammatory types, thus the induction of these cytokines by IC and their effect on the clinical variations could contribute to better understanding of such variations.

Microfilaricidal drugs are thought to somehow involve the immune system in their killing mechanism. It has been proposed that their action involves unmasking of previously hidden parasite antigens and thus stimulating antigen presentation to the effector cells.
The present study is an attempt to gain better understanding to the role of the immune system in the development of pathology and the varied clinical presentations seen in onchocerciasis patients. It is also intended to identify any role the immune system might play in the microfilaricidal action of ivermectin (Mectizan)®, and to investigate if the host genetic factors might mediate these immune variations.
Objectives

- To better define and recognize the reactive onchodermatitis (sowda) form of the disease, thus determining the prevalence of this severe form of onchocercal dermatitis in different endemic areas of Sudan.
- To develop a comprehensive definition of this condition.
- To characterize humoral and cellular immune responses in onchocerciasis patients with different clinical presentations.
- To study the effect of ivermectin treatment in these patients and to see if the immune system mediates the outcome of treatment.
- To investigate the effect of IC from *O. volvulus*-infected patients on the development of dermatopathology.
- To investigate the associations of FcγRIIa polymorphism with the varied clinical pictures seen in clinically distinct groups of *O. volvulus*–infected patients.
MATERIALS AND METHODS

For information regarding the materials and methods used for this thesis please see the materials and methods sections of the respective paper.
**Results and Discussion**

**Paper 1**

Onchocerciasis is regarded as a disease where the range of clinical presentations is paralleled by a spectrum of different immune responsiveness; reactive onchodermatitis (ROD) or sowda representing one pole of this spectrum. Recognition of this form of dermatitis is central to studies on clinical, therapeutic and pathogenic aspects of the disease. The present study was carried out in Sudan- a country known to carry a high prevalence of ROD with the aim to determine the prevalence of this unique form of onchocercal dermatitis in different endemic areas, and also to develop a comprehensive definition thus assisting to standardize data regarding this condition. ROD has a number of defining characteristics, the definition is divided into those characteristics that were almost always present (likely characteristics) and other features that may sometimes be present. Two specific cases were then selected from this analysis exemplifying the definition developed; these cases covered the major characteristics of ROD as was redefined by this present study. The parasitology in ROD is characterized by negative, or very low, skin-snip mf load. The condition was extremely pruritic and both femoral and inguinal lymph nodes on the affected side were prominently swollen. Extensive excoriations were present on the affected areas, as well as bleeding puncture marks due to self inflicted trauma consequent to the intolerable irritation. One of the typical cases reacted severely to treatment with Mectizan®.

Three major foci of this disease exist in the country which are geographically disparate and have different levels of endemicity and clinical patterns. A typical blinding savannah form of the disease is common in the south, with generalized onchocercal dermatitis (mostly mild form) with high mf loads and palpable nodules prevalence. In contrast to the northern and eastern foci where the disease is associated with severe dermal pathology, low mf loads and less palpable nodules. Nevertheless, cases of ROD were detected, in all endemic
areas of Sudan, but the prevalence of ROD was strikingly higher in the hypo-endemic area (the eastern focus) compared to that of the hyper and meso-endemic areas.

The characteristics collated here from the experience of onchocerciasis in Sudan, provide, for the first time, a practical definition of this form of the disease: a definition that is likely to be applicable to other areas of the endemic disease, and to aid the elucidation of the immunopathogenesis of this form of the disease and its relationship to spectrum on its skin related manifestations. The very low mf load seen in this unique form, might have resulted from an active *in vivo* destruction of the dermal mf, leading to significant burdens of dying or non-motile parasites that escape detection due to their damaged state (Mackenzie *et al.*, 1985). The skin changes in ROD are most commonly localized to a single limb or area of the body, and a darkening of the affected area almost always occurs, hence the name ‘sowda’, an Arabic word meaning ‘looks dark or black’ (Connor *et al.*, 1983; Fawdry, 1957). ROD cases are seen in both males and females; most cases encountered in the present and other previous studies are young, mostly in their teen years (Ghalib *et al.*, 1987; Mukhtar *et al.*, 1998). The finding that one focus (hypo-endemic) is interestingly characterized by a high prevalence of ROD could partially be due the very short transmission (maximum of 4 weeks per year or every other year) period in this area leads to an infrequent exposure, thereby rendering the immune responses of these infected individuals to react more vigorously (Mackenzie *et al.*, 1985). However, other factors might contribute to variations in the prevalence of ROD; the black fly vector could be affected by the ecology of the area. The parasite’s strain and the relevant burdens of its endosymbionts (*Wolbachia*) (Higazi *et al.*, 2005; Higazi *et al.*, 2001). Moreover, as the different investigated foci are populated by different ethnic groups, host genetic factors may also be responsible for this variation. An important aspect of ROD is the severe side reactions to treatment with ivermectin which was not related to the pharmacokinetic profiles of ivermectin in those reacted severely to the drug and those who
didn’t (Baraka et al., 1995b). This raises the likelihood that immune responses in these patients are underlying these post-treatment reactions. ROD patients have increased capacity to kill mf, and thus more bacterial (Wolbachia) products to be released per parasite (Keiser et al., 2002).

We conclude that ROD is a clinically important form of onchocerciasis, which needs further investigation regarding both clinical aspects and the definition of the underlying pathogenic mechanisms. The severity of the condition necessitates that patients be treated in a timely manner treatment: these individuals have often been misdiagnosed as suffering from conditions other than onchocerciasis (Mackenzie et al., 1985). In our following research papers we tried to better understand the immunologic basis of this condition (paper II, III and IV). Then we also investigated the role of genetic polymorphism in one the genes encoding receptors associated with innate as well as adaptive immunity (paper V).

**Paper II & III**

The basis for clinical variations in onchocerciasis is believed to primarily involve an intimate relationship between the parasite and the host’s immune system. *O. volvulus* mf has long been known to be involved in the acute papular dermal responses and the typical punctuate keratitis seen in the corneas of the affected people (Mackenzie et al., 1985; Williams et al., 1987). Thus, we hypothesized that, the worsening clinical presentation of chronic papular dermatitis and other severe changes seen in onchocerciasis involve inflammation related to mf and their destruction. In these present studies, we tried to test the role of the likelihood that increasing cycles of infection involving mf are central to the mostly seen reactions. We investigated the significance of the immune responses directed against mf in onchocerciasis patients presenting with two major forms of the dermal disease: mild or asymptomatic, and severe dermal manifestations including ROD. We examined these immune responses to determine
the significance of reactions to *O. volvulus* mf in the pathogenesis of disease in these two clinically different groups. Since we believe that the underlying mechanisms associated with this disease mostly involve the immune system, we also hypothesized that immunocompetence may be important for the effective action of the microfilaricidal drug currently in use Mectizan ® (ivermectin). Or it may be an after effect, as the immunosuppression in mf positive patients tends to greatly be reduced following treatment with this microfilaricidal drug.

Our results from paper II showed that higher mf loads were seen in the mild skin disease group, whereas markedly lower loads were evident in the one with the severe dermatopathology. Thus, there was a significant inverse relationship between the mf loads and the severity of clinical manifestations. Serum samples from this latter group also mounted strong cell adherence to the surface of mf, unlike the group with mild dermatopathology who showed only minimal cytoadherence activity in the cell adherence assay set up system. Interestingly, a positive relationship between the ability to mount an active cell-adherence response and an active cell-mediated response was seen. These responses were found to be inversely correlated to the mf loads; the lower the mf load the greater the cytoadherence activity and cell proliferation responses. The cell-mediated responses of some patients were also assayed following treatment with ivermectin, and responses were found to be increased in all cases, however, most significantly seen in those with the mild form of the disease.

The positive correlation between immune responses and clinical severity seen (paper II) might reflect an increased inflammation associated with increased *in vivo* mf destruction and thus, a decreased detectable mf load paralleling an increased severe dermatopathology. Patients with this latter condition including ROD might have higher burden of dying or non-motile parasites that are not detected in skin-snip assay, which only demonstrates live parasites. Immunosuppression towards mf has been suggested earlier
(Elkhalifa et al., 1991) and the poor cytoadherence to O. volvulus mf in the mild dermatopathology group may reflect that the antibody-mediated immune responses in these individuals are ineffective at destroying mf \textit{in vivo}. This may in part explain their quiescent state compared to the other group. However, this could be looked at as an immune evasion by the parasite. It has been suggested that, one factor contributing to this hyporesponsiveness is alteration of APC function (Hoerauf et al., 2005; Semnani et al., 2004). More recently, this dysregulation was demonstrated to be associated with diminished CD4$^+$ T cell production of IFN-gamma and IL-5. This is in association with diminished expression and functions of TLR, thus lends a likely consequence of chronic Ag stimulation and could serve as a novel mechanism underlying the dysfunctional immune response in filariasis (Babu et al., 2005).

Ivermectin treatment appeared to enhance cell-mediated responses a finding in line with previous studies (Soboslay et al., 1994). Immune mechanisms may be involved not only in the initial induction of mf killing, but also in maintaining an ongoing process of mf destruction. Thus, resurgence of mf loads in previously treated individuals might be influenced by the quality of immune responses. This rings the bell to wonder if immunocompetence is needed to curtail the infection for a longer time that led us to perform our third study. We therefore here, investigated the assumption that immunocompromised individuals are less effectively responding to the microfilaricidal agent (ivermectin). A significantly higher mf load in this latter group of patients was found as compared to the control group which was able to maintain very low levels of dermal mf for an extended period of time. Also, there were lower proliferative responses to stimulation with onchocercal antigens in the early returning groups. Immune, non-specific and drug induced destruction of mf can all occur at different time during the infection. Moreover, this study supported our previous assumption and findings in paper I, that ROD patients are usually prone to develop severe post-treatment reactions due to their heightened or vigorous immune responses thus,
enhancing more \textit{in vivo} mf killing. This necessitates more caution should be paid when treating this group of patients. The reason for reduced ability of Mectizan\textsuperscript{®} to maintain low levels of dermal mf, and diminished ability to reduce the major clinical pruritus, could only be speculated upon. Of note, however, most of the returnee harbor multiple nodules which provide sources of new dermal mf. Another explanation could be due to the weakening effect of the drug or may, as supported by our findings, reflect an inability of the host’s immune system to contribute to drug-initiated microfilarial destruction.

Our findings differ from other previous work that annual treatment seem to be suffice to curtail mf loads for much longer time before resurgence of parasites (Awadzi \textit{et al}., 1989; Brieger \textit{et al}., 1998; Brown & Neu, 1990; Ette \textit{et al}., 1990). Noteworthy, this phenomenon could have a draw back to the control programs, as disappointing this small proportion of patients with the drug effectiveness, might also discourage the population at large.

Our findings also underline the significance of including clinical evaluation in epidemiological mapping for the disease prevalence rather than solely depending on parasitological tools in an agreement with the pertinent recommendations raised earlier (Ghalib \textit{et al}., 1987; Mukhtar \textit{et al}., 1998).

Finally, the observations in the present study supported the intriguing possibility that there is an active involvement of the immune system in the mechanism of action of ivermectin, as we hypothesized in our previous two studies. This concept, however, needs and should trigger further investigations.
Figure 2 The left picture shows a mf lying in a dermal lymphatic, but free of any cellular reaction. The basal layer of the epidermis is somewhat disrupted and there proliferation of the melanin containing cells (hyperpigmentation). The right one shows a severe case of sclerosing keratitis. The cornea is replaced by opaque scar. These slides were kindly provided from C.D Mackenzie, Michigan State University, USA

Paper IV

Increased levels of circulating (IC) containing parasite antigens have been associated with the disease severity and inversely with dermal mf load, suggesting that IC might take part in the in vivo mf destruction postulated in immune competent patients (Paper I, II and III). TNF-α and the closely related cytokine IL-β are predominantly monocyte-derived pro-inflammatory, and are both counteracted by regulatory substances also produced by monocytes a concept that has been efficiently used in the treatment of e.g. rheumatoid arthritis (RA) (Bresnihan et al., 1998; Moreland et al., 1997). IC-induced cytokine production might be pathophysiologic mechanisms in O. volvulus infection; however, the nature of the cytokines response induced has not been reported previously. We here for the first time investigated the cytokine-inducing effects of the circulating IC from clinically well characterized Sudanese patients with onchocerciasis, focusing primarily on the production of the central pro-inflammatory cytokines TNF-α, IL-1β and their physiological antagonists TNF-Rp75 and IL-1ra together with the anti-inflammatory IL-10. We found that polyethylene glycol (PEG) precipitates from the plasma of infected patients induced significant production of the cytokines TNF-α, IL-1β,
and also IL-10, following stimulation of PBMC cultures from healthy individuals. Interestingly, the induced levels of IL-1ra, the endogenous competitive blocker of IL-1 receptor in contrast are down regulated by PEG precipitates derived from *O. volvulus*-infected patients as compared to precipitates from Sudanese healthy controls. Our findings also confirmed reports from previous studies, that circulating C1q binding IC were high among patients with *O. volvulus* infection (Chandrashekar *et al.*, 1990; Semnani *et al.*, 2004; Sisley *et al.*, 1987). This present study also showed that plasma levels of IgG correlated positively with induced levels of TNF-α, IL-β and IL-10, but negatively with IL-ra, whereas IgA levels correlated with inflammation measured as CRP.

Our findings of increased levels of IL-10 as well as of the pro-inflammatory cytokines induced by PEG precipitates from onchocerciasis patients are in an agreement with the notion that the latter cytokine dominates during the early filarial infection (Babu & Nutman, 2003) with inflammation later being down regulated by IL-10. Moreover, the IC-induced production of IL-10 has been shown to significantly influence immune responses in animal models. The susceptibility to infection was found to be increased when neutralizing IC-induced IL-10 production (Shanley *et al.*, 1995).

The decreased production of IL-1ra as compared to IL-1β is an intriguing finding. IL-1ra, like IL-1β is mostly produced by monocytes and macrophages, and has powerful capacity to counteract the pro-inflammatory effects of IL-1β. A central role for IC-induced mechanisms in the development of keratitis in *O. volvulus* infection is implicated by the fact that both antibodies and FcγR are needed for the development of corneal pathology in *Onchocerca*-infection (Hall *et al.*, 2001; Hall & Pearlman, 1999). Our findings therefore, imply that IC-or *O. volvulus* antigen-induced suppression of IL-1ra production might be pivotal in onchocerciasis-associated pathology and moreover, convey a new knowledge of potential value for the understanding of the clinically important immune suppression. Thus,
therapeutic supply of IL-1ra might be beneficial in local or systemic treatment of *O. volvulus*-associated keratitis or in the treatment of severe ROD-associated intolerable unremitting itching.

**Paper V**

Adequate activation threshold of various cells in our immune system depends to a large extent on immune activation and inhibitory receptors. We have attempted to address and better understand the long standing interplay between infection status and the balance between the immune responsiveness and modulation. Some functional assays were carried out that strongly suggested the intimate involvement of immune system in the development of the varied clinical manifestations (paper II, III and IV). However, the propensity of some patients to develop ROD might more clearly be understood by identifying some genetic characteristics among those affected. Indeed genetic variations in FcγRs constitute an important determinant for host defense capabilities. To our knowledge this is the first paper to describe any association of the FcγRIIa polymorphism with the clinical variations seen in *O. volvulus*-infected patients. Our results showed marked variation in the genotype frequencies among the clinically different groups, tribes and age groups, as this polymorphism represents itself as an inherited risk factor in the pathogenesis of onchocerciasis. The R 131 genotype was found to dominate among patients with severe dermatopathology including ROD compared to those with mild form of the disease. Moreover, allelic frequency analysis suggests that this is due to the negative and possibly protective effect of the H allele in those with the mild form of the disease. Interestingly, when taking into account differences in ethnicity (taking the Fulani tribe as the reference category due to their higher number), we found increased risk of developing this severe form among the Masaleet tribe. This tribe emigrated recently from a non endemic area in western Sudan (Darfour) whereas; the Fulani tribe originally came from West Africa (40-50 years ago) where infection with onchocerciasis is very common. Age also
seems to have an impact on the likelihood of developing the ROD form. It was found to be more frequent among the youngest group of patients, confirming the observation that was made in paper I. Differences in disease patterns in different age groups reflect a rich mix of environmental factors and may also reflect population changes in genetic factors. Although a clear association with disease severity was thus identified, we found no association between this polymorphism and the risk of contracting the infection. This finding suggests that the polymorphism is not related to protection from the infection, but possibly protective from developing the severe dermatopathology.

Patients homozygous for the R$^{131}$ are at higher risk for serious infection with encapsulated organisms and gram negative bacteria including *Wolbachia* and for the impaired IC removal (Karassa et al., 2002). In our previous paper we demonstrated high levels of IC which were also found to induce TNF-a, and IL-1β but to suppress IL-1ra thus further maintaining the inflammatory consequences. This might thus partially explain the reactive onchodermatitis seen in these patients with dominant RR genotype. Because H allele is associated with mild form of dermatitis, this might be inferred as being protective from the antibody-dependent enhanced immunopathological consequences seen in the group with the severe form, who demonstrate ongoing in vivo mf destruction with more bacterial (*Wolbachia*) products released as suggested in paper II.

The data described are in accordance with a report by Shi et al. (Shi et al., 2001) showing that the FcγRIIa-R$^{131}$ allele, which also has a high affinity for CRP, is associated with protection against high parasitemia in *P. falciparum* infection in Kenya. Of note, in our previous paper, we also found higher levels of CRP in onchocerciasis patients. Those findings together with ours, suggest that the binding of CRP to FcγRIIa might also play a role in the development of pathology in onchocerciasis patients. It is tempting to speculate that selective pressures influenced the worm to decorate itself with CRP thus ensuring ligation of FcγRIIb
(inhibitory) in its vicinity. This could allow the parasite to protect itself from local immune responses. Alternatively, the host might have responded by selecting an R variant of the Fc$\gamma$RIIa that could bind the CRP coating and induce an inflammatory response.

**CONCLUDING REMARKS**

ROD, with its severe and debilitating nature, is clinically the most important form of onchocerciasis. Due to diagnostic reasons, it has been often ignored and is thus appears to be more common than has previously described. This form of the disease has special characteristics and important information that could greatly assist the general understanding of the disease as a whole. Thus, it is important that ROD is accurately reviewed, characterized and identified in the field situation, and is included in both epidemiological and pathogenesis studies. The epidemiological aspects are especially important in geographical areas that have been classically defined as hypo-endemic. Including ROD cases in the prevalence figures for these areas will often upgrade the status to a higher endemicity level, thus allowing for their inclusion in the treatment control programs. This is also clinically important as these patients are always desperately in need of treatment as they suffer more than any other group of onchocerciasis patients.

Cell-mediated immunity (CMI) and antibody-mediated immunity (AMI) responses are known to play a significant role in the development of dermal pathology. There is a direct relationship between increased cell cytoadherence/cell-mediated immunity and increased severity of dermatopathology; however, the detailed characteristics of the events and consequences associated with the death, breakup, and removal of microfilariae are still unclear. Likewise, effective anti-filarial chemotherapy also appears to intimately involve the immune system, either as consequence of treatment or through being directly involved in the drug’s action. Thus, a patient’s immunocompetence may be essential to achieving effective
and efficient responses to drug treatment, and correspondingly immunologically compromised onchoderciasis patients may not respond effectively to anthelminthic treatment.

Our findings suggest novel beneficial therapeutic strategy by supplying IL-1ra to onchoderciasis patients, e.g. as topical ocular treatment or as skin application to relieve the intolerable itching presented with the severest form of the disease ROD.

The propensity to develop ROD appears to involve marked variation in the genotype frequencies among the clinically different groups, tribes and age groups.

The selective pressures are more likely to have operated through the influence of disease severity rather than initial infection.

**PERESPECTIVES**

- Further studies to elucidate the role of the immune system in the action of ivermectin?
- How does this phenomenon contribute to the concept of “drug resistance” in onchoderciasis? *Resistance may be due to poor immune responses?*
- How do these immune responses affect the adult worms (an important target for treatment)? Which are also contributing to the cumulative effects of the long term treatment on the adult worms?
- Other candidates for the clinical variations? Study polymorphisms in TLR2 and TLR4 among the different groups of patients representing different ethnic groups.
- Functional studies of IC from Onchocerca-infected patients, as well as investigations of the effects of purified and different *O. volvulus* derived antigens on IL-1ra production, might yield additional knowledge of the pathogenesis and potential therapeutic significance for patients with different clinical forms of the disease.
Figure 3 Summary of our view to the consequence of events in an *O. volvulus* infection:
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