academicJournals

Vol. 8(4), pp. 39-46, April 2016
DOI: 10.5897/JPVB2016.0233
Article Number: 515A9B058365
ISSN 2141-2510

http://www.academicjournals.org/JPVB

Journal of Parasitology and Vector Biology

Full Length Research Paper

Influence of infective stage (L3) dose on the outcome of microfilaremia, peripheral white blood cells and humoral immune response in *Loa loa* experimentally infected *Mandrillus sphinx*

J. P. Akue[1]*, P. Makouloutou[1,2], R. M. Moutsimbi[1], H. Moukana[1] and E. Leroy[1]

[1]Departement of Medical Parasitology, Centre de Recherches Médicales de Franceville, Gabon.
[2]Institut de Recherche en Ecologie Tropicale, Centre National de la Recherche Scientifique et Technologique, Gabon.

Received 1 January, 2016; Accepted 8 April, 2016

Analyses were performed to assess how the dose and frequency of administration of the natural infective stage (L3) affects events in the peripheral blood of an infected host. A blood sample was collected from different mandrills (*Mandrillus sphinx*) infected once with 1000 L3 *Loa loa*, 250 L3 four times, 100 L3 once and 25 L3 four times. Enzyme-linked immunosorbent assay (ELISA) was used to measure levels of microfilaria-specific IgG and Western blotting to identify antigens. Fluctuation in the number of neutrophils, eosinophils and monocytes was seen during prepatency and patency in relation with some L3 doses. The density of microfilaria in the peripheral blood showed that animals that received 1000 L3 had the highest density of microfilaria as compared to those that received 250L3 ×4, 100L3 ×1 or 25L3 ×4. In contrast, the microfilaria-specific IgG level was higher in animals that received doses of 25 L3 ×4 as compared to those that received 1000 L3 ×1 ($p< 0.001$) but comparable with the level in the groups that received 250 L3 ×4 or 100 L3 ×1 ($p>0.05$). Identification of microfilaria antigens recognized by this specific IgG on Western blotting revealed the presence of 23-, 20- and 14-kDa antigens specifically in animals that developed patent infection. These results suggest that the regimen of L3 administration may have an effect on the level of humoral immune response and to some extent on the density of microfilaria.

Key words: *Loa loa,* Helminths, larvae, immunology, primate.

INTRODUCTION

Loa loa is becoming an emerging disease as more cases are reported throughout the world (Antonori et al., 2012). Therefore, searching for the factors controlling microfilarial load (Gardon et al., 1999) may be valuable in order to develop alternative control strategies. Transmission, which is based on infective stage (L3) has been shown to

*Corresponding author. E-mail: jpakue@yahoo.fr. Tel: 241 67 70 92. Fax: 241 67 72 95.

induce changes in the humoral and cellular immune response. However, neither the dose nor *the frequencies are known in natural conditions. Although, there are several animal models of filarial, L. loa* presents clinical specificities such as encephalitis (Carme et al., 1991) that require particular attention. This clinical expression suggests that this parasite should be studied with a homolog parasite.

Mandrillus sphinx, a commensal primate species living in the same area, is receptive to *L. loa* (Pinder et al., 1994). It has also developed a state of amicrofilaremic common in humans from this region (Dupont et al., 1988). Furthermore, it is phylogenetically closer to humans than the model based on other heterologous species. An experimental model provides the opportunity to study a controlled infection where the time and the number of infective larvae are known in contrast to natural infection where these parameters are unknown. An earlier study with this model used irradiated L3and did not address the specific question of L3 dose and frequency of inoculation (Akue et al., 2001) but found a relation between 20 kDa of microfilaria (mf) and mf appearance. The present study addresses the issue of the natural infective stage (L3) dose and inoculation frequency on mf load in peripheral blood of domestic-born and -bred *Mandrillus sphinx* from the primatology center at the International Center for Medical Research of Franceville (CIRMF). At the same time, this study examined the level of humoral immune response against microfilarial antigens in this experimental condition. Therefore, the aim of this study was to measure IgG, microfilaria, and white blood cells under stimulation with different quantities of natural infective larvae.

MATERIALS AND METHODS

Ethics statement

Animals were handled according to European directive CEE 10/63 and the authorization of the Gabonese Government (reference RG/MINEF/00468); efforts were made to minimize animal suffering.

Mandrillus sphinx

This study is based on the use of serum samples and data were recorded on mandrills infected experimentally and followed up for several years. A group of eight male and female *M. sphinx* from CIRMF's breeding colony was housed in outdoor group cages. Baseline clinical and biological examinations were performed and showed no sign of filarial infection. These animals were split into four groups: one group was inoculated with 1000L3 at once (two animals: 10A1&5F), one with 250L3 at four different times (two animals: 12I&5A2), one with 100L3 inoculated once (two animals: 16C&24), and another one inoculated with 25 L3 at four different times (two animals: 5A3&6C). examination of animal consist of direct examination of each blood sample from the mandrill, followed by a concentration technique for parasite, blood cells count and biochemistry such as blood sugar, creatinine, urea, transaminase were also performed. The age of animal varied between 7 and 10 years and the control group is mandrill that is not infected.

Loa loa infective stage (L3)

L3 is the stage that ensures the efficacy of transmission of the filarial *L. loa* from the natural host (*Chrysops*) to humans. L3 larvae were therefore purified from naturally infected *Chrysops* flies by maceration, using the Baerman technique, as modified by Wahl et al. (1995). L3 were selected using a Pasteur pipette and transferred to a fresh solution of phosphate-buffered saline (PBS).

Infection of mandrills with *L. loa* L3 and follow-up

The number of larvae to be inoculated was resuspended in sterile PBS solution and pulled up into a 1-ml syringe with a 21-gauge needle. These larvae were inoculated subcutaneously on either the right or left side of the chest. During follow-up, the animals were systematically bled under anesthesia at different time points before, during and after the infection. Since the aim was to follow up the effect of a natural L3 dose on different biological parameters, but not a vaccination per se, a challenge infection was not carried out on the animals.

Microfilaremia detection and plasma collection

From bled animals at different time points before, during and after infection, 6 ml of total uncoagulated blood were collected and 4 ml were centrifuged to collect plasma. Plasma was kept at −70°C until used and peripheral blood cells were counted on 1 ml of uncoagulated blood, and 1 ml of uncoagulated blood collected on EDTA was used to determine the mf quantity using a concentration technique. Briefly, 1 ml of total blood mixed with 9 ml of PBS and 2% saponin were centrifuged for 10 min at 3000 g. The pellet was analyzed under the microscope to identify mf.

Microfilaria antigen preparation

Mf were obtained from heavily infected patients and purified on Percoll gradients, as described previously (Van Hoegaerden and Ivanoff, 1986). Mf were disrupted by sonication in 10 mM Tris-HCl, pH 8.3, containing protease inhibitors. Antigens were extracted with 1% sodium deoxycholate for 1 h at 4°C. The extracts were centrifuged at 10,000 *g* for 10 min, and the soluble supernatant was aliquoted and stored at -20°C. The protein content was measured using the Biorad method (Bradford, 1976).

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed. Briefly, detergent-soluble extracts of *L. loa* mf were diluted in carbonate-bicarbonate buffer, pH 9.6 and 100-µl volumes were distributed into the wells of microtiter plates (Immunolon II, DynatechLaboratories, Chantilly, VA, USA) at 10 µg/ml. After an overnight incubation at 4°C, the plates were washed three times for 10 min at room temperature with 50 mM Tris-HCl, pH 7.4, 200 mM NaCl and 0.05% Tween 20 (TBST). The plates were then blocked for 1 h with TBST-5% bovine serum albumin (BSA), and the washing step was repeated. Mandrill plasma diluted at 1/400 in TBST-1% BSA was added for 1 h, and the washing step was repeated and followed by incubation with mouse anti-human IgG Fc-specific alkaline phosphatase conjugated (Sigma, St Louis, MO, USA) for 1 h. After washing the plates, the reactions were detected using p-nitrophenyl phosphate (Sigma) diluted in diethanolamine buffer, pH 9.8, and the optical density (OD) was read at 405 nm using an LP 200 micro-ELISA reader (Diagnostic Pasteur). Positive and negative controls were used: positive animals previously infected by *L. loa* and negative animals not

infected or before their infection. The cut-off value for ELISA is defined as the optical density (OD) before infection of the animals tested plus one standard deviation (OD: 0; 340), however data were presented as crude OD to show the real kinetic on antibody from day -126 to the end of experiment.

SDS-PAGE and Western blotting

As previously described (Akue et al., 2001), soluble *L. loa* mf antigens were separated by SDS-PAGE under reducing conditions on 12.5% acrylamide mini-gels, with a discontinuous buffer system (Laemmli, 1970). A total of 100 µg of protein was analyzed in a continuous well. Separation was optimal over the 97- to 10-kDa molecular range. Following electrophoresis, proteins were transferred onto nitrocellulose paper (NCP) by electrophoresis in a buffer containing 0.25 M Tris-HCl, 0.192 M glycine, 20% methanol, pH 8.3 (Towbin et al., 1979). The NCP was stained with Ponceau (Sigma) to visualize the bands. Then, the paper was blocked for 1 h in Tris-buffered saline, 0.05% Tween 20 (TBST) with 3% BSA. Following three washes in TBST, each strip of NCP was incubated overnight at 4°C with plasma from individual mandrills at different time points of the follow-up and diluted at 1/400 in TBST 1% BSA. The strips were washed and then incubated for 1 h with mouse anti-human IgG alkaline phosphatase diluted at 1/1000 in TBST 1% BSA. This was followed by another washing step and incubation in the substrate solution containing 5 bromo-4-chloro-3 indolyl (BCIP, 0.3 mg/mL) and Nitroblue-Tetrazolium (NBT, 0.15 mg/mL) in 1 M Tris-HCl, 500 mM $MgCl_2$ buffer for visualization of bound antibodies.

Statistical analysis

The mean value of each parameter was calculated ±SD (standard deviation). A Mann-Whitney U-test was used to compare the levels of white blood cells (WBCs), specific IgG and a Fisher exact test was used for microfilaria density. A p-value < 0.05 was considered significant.

RESULTS

Follow-up of peripheral white blood cell counts

Total WBCs remained stable at all doses except at a dose of 250L3×4 where fluctuations were observed. At this dose, the numbers increased significantly during prepatency and patency periods as compared to the basic level before infection (6850±1138 vs. 10,220±3545; 6850± 1138 vs. 10,444 ± 1565; p = 0.0227 and p = 0.0018, respectively). For lymphocytes, no differences were seen at any dose or time. Monocytes at a dose of 25 L3 ×4 decreased significantly in number during prepatency and patency (156±109 vs. 51± 55, p = 0.0074 and 156 ±109 vs. 56±79, p = 0.054, respectively). Similarly, at a dose of 1000L3 ×1, a significant decrease was also observed during patency (213 ± 110 vs. 72 ± 105, p = 0.0368). For neutrophils, in the group that received 25L3 ×4, a decrease was observed during the prepatency period as compared to the initial level (4128± 2706 vs. 1875± 2360;p = 0.0295). In contrast, the level of neutrophils increased in the mandrills that received 250L3 ×4 during patency as compared to their initial level before infection (2033± 1303 vs. 4870± 1539; p = 0.0080). For eosinophils, many fluctuations were observed. Within groups, there was a significant increase in the level of eosinophils during prepatency and patency in the group that received 25 L3 ×4 (48vs 656, p=0.0046; 196 vs. 656, p = 0.0218, respectively). Similar observations were made within the group that received 100L3 ×1 when comparing their level before infection and in the prepatency (0 vs. 582, p = 0.0149) or patency period (0 vs. 720, p = 0.0092). Between groups, the levels of eosinophils were significantly higher at a dose of 250L3 ×4 as compared to the level at a dose of 25 L3 ×4 during prepatency (768± 41 vs. 253 ± 245, p= 0.0113) as well asat a dose of 1000L3 ×1 during prepatency (768 ± 411 vs. 270± 205, p = 0.0048) and patency (634± 411 vs. 249 ± 220, p = 0.0334). At a dose of 100L3 ×1, eosinophils were significantly elevated during prepatency as compared to the level seen at a dose of 25 L3 ×4 (671± 449 vs. 253 ± 24, p = 0.0373) and also significantly elevated as compared to that seen at 1000L3 ×1 during prepatency (671 ± 449 vs. 270 ± 205, p = 0.0305) and during patency (703 ± 530 vs. 249± 220, p = 0.0186). Interestingly, even at a dose of 25 L3 ×4, the level of eosinophils was significantly higher during patency than the level seen with the 1000L3 ×1 dose (707± 438vs 249 ± 220, p = 0.0184) (Table 1).

Comparative density of *Loa loa*microfilaria according to dose and frequency of inoculation

Research on mf was conducted on infected animals using the concentration technique. It appeared that there was no significant difference with the time it took mf to appear in peripheral blood: 154, 156, 156 and 176 days after infection with 1000L3 ×1, 250 L3 ×4, 100L3 ×1 and 25L3 ×4, respectively, although that time was slightly longer with the latter dose as compared to the others (Table 2). Furthermore, mf density varied at the individual level for all animals, with a trend toward more elevated mf density for the 1000L3 ×1 dose followed by 100 L3 ×1, 250L3 ×4, and 25 L3 ×4, but none of these differences reached the statistically significant level (p>0.05). Interestingly, when frequency of inoculation was considered, it appeared that animals receiving sequential doses (four times) had the lowest level of mf, whereas those receiving a massive single dose had the highest density of mf. Similarly, the time it took mf to appear was in general delayed in the animals that received four inoculations. After the peak of mf (days 145 and 230), the fluctuation observed after day 245 did not significantly change the level of mf during this phase.

Level of anti-microfilarial response as a function of L3 dose and frequency of administration

A longitudinal study was conducted on the plasma of each infected mandrill that received a different dose of

Table 1. Fluctuations of total white blood cells before infection, at prepatency and patency (mean±SD)

Cells type	Before infection				Prepatency				Patency			
	10A1/5F	12I/5A2	16C/24	5A3/6C	10A1/5F	12I/5A2	16C/24	5A3/6C	10A1/5F	12I/5A2	16C/24	5A3/6C
Total white blood cells	9125±2705	6850±1138	10557±4050	9475±2475	8800±4107	10220±3545	8989±2188	9290±1692	9508±3168	9508 ±1565	10222±3954	8843±1479
Neutrophils	3785±1928	2033±1303	5982±4442	4128±2706	2891±1623	4117±3641	3447±1668	1875±2360	4097±1690	4870±1539	3979±4036	3327±1419
Eosinophils	211±179	360±215	77±97	138±179	270±205	768±411	671±449	253±245	249±220	634±411	703±530	707±438
Lymphocytes	4895±2550	4009±1142	3991±1160	5041±2082	5392±3213	5234±1647	4761±1516	5342±2071	5012±1782	4895±880	5291±1066	4750±539
Monocytes	213±110	110±64	207±216	156±109	123±155	98±62	83±68	51±55	72±105	53±66	55±102	56±79

L3. It was shown that the IgG level (Figure 1) progressively rose from day 85 during the prepatency period till the patency period (days 148–174) when they reached these maxima according to the L3 dose. The specific IgG response remained low in animals that received 1000L3 ×1 (Figure 1, curve 1) as compared to all other animals ($p< 0.001$). In contrast, animals that received 25 L3 ×4 (Figure 1, curve 4) had the highest level of IgG as compared to those that received a dose of 250 L3 ×4 (Figure 1, curve 2) and 100 L3 ×1 (Figure 1, curve 3), although these differences are not statistically significant ($p> 0.05$).

Antigen recognition by microfilaria-specific IgG according to L3 dose

Longitudinal analysis of the recognition of mf antigens by infected mandrills was carried out based onthe L3 dose. These analyses showed that infected mandrill recognized a larger number of antigens with a molecular weight varying from 104 to 10 kDa. The frequency of molecule recognition from 100, 66, 45, 30, 22, 14 and 11 kDa was very high in all infected microfilaremic animals despite different doses (Figure 2), while animals that did not develop patent infection characterized by mf in peripheral blood recognized only molecules above 30 kDa (data not shown). These observations were independent of the dose received. In order to check whether the pattern of low-molecular-weight recognition was related to parasitological status beyond one year, *M. sphinx* that were previously infected and followed up for seven years were used to analyze their reactivity against mf antigens. Animal 2A, which reached the highest mf density of 56 mf/mL and remained mf-positive for years, was examined at five time points for its reactivity. It was shown that this animal at any time point (Figure 3, lines 1 to 5) recognized 14- to 20-kDa molecules. The second animal (mandrill 19) followed up for seven years with the highest mf level – 31,000 mf/mL remained microfilaremic during this time and the mf level was analyzed at five time points. The pattern of antigen recognition was also dominated by the recognition of the 20-kDa molecules at all-time points (Figure 3, lines 6 to 10). The third animal (mandrill 20) had the highest mf level at 17 mf/mL once but remained consistently low for mf for 5 years when tested for antigen reactivity at time points when it had a low level of mf (Figure 3, lines 11 to 12). Different antigens were recognized including the 20-kDa molecules, but the same animal (mandrill 20), after the disappearance of its infection (no more microfilaria 27/1/94 = 0 mf/ml), had no more antibodies against the 20-kDa molecules at any time point after this disappearance of infection both 6 months (Figure 3, line 14) and 1 year later (Figure 3, line 15). The reactivity of all these animals was compared to the reactivity of an uninfected animal (Figure 3, lines 16 to 17).

DISCUSSION

The effect of inoculation of different L3 numbers and their sequential administration on peripheral white blood cells, mf appearance, specific IgG level, and their target antigen on mf in an experimental model of *L. loa* were analyzed. A cellular immune response was reported previously and showed a transient strong immune response followed by a state of unresponsiveness in microfilaremic animals (Leroy et al., 1997). Another study investigated protective immunity in this model using irradiated larvae (Akue et al., 2001), and the impact of vaccination with irradiated larvae on microfilaria and the humoral response were shown in comparison with normal larvae. In the absence of a small animal model, a primate model was used in loiasis to study immunological, hematological and parasitological parameters during the course of infection by *L. loa* in rhesus monkeys (Dennis et al., 1993) and *M. sphinx* (Pinder et al., 1994). However, no single study has addressed the issue of L3 dose and frequency specifically on the expression of

Table 2. Appearance of mf in the peripheral blood according to L3 dose.

Animals	L3 Dose	Patency (days)	mf density (range mf/ml)
10A1/5F	1000×1	154	929 (0-11700)
12I/5A2	250×4	156	49 (0-567)
16C/24	100×1	156	175 (0-2300)
5A3/6C	25×4	176	21 (0-105)

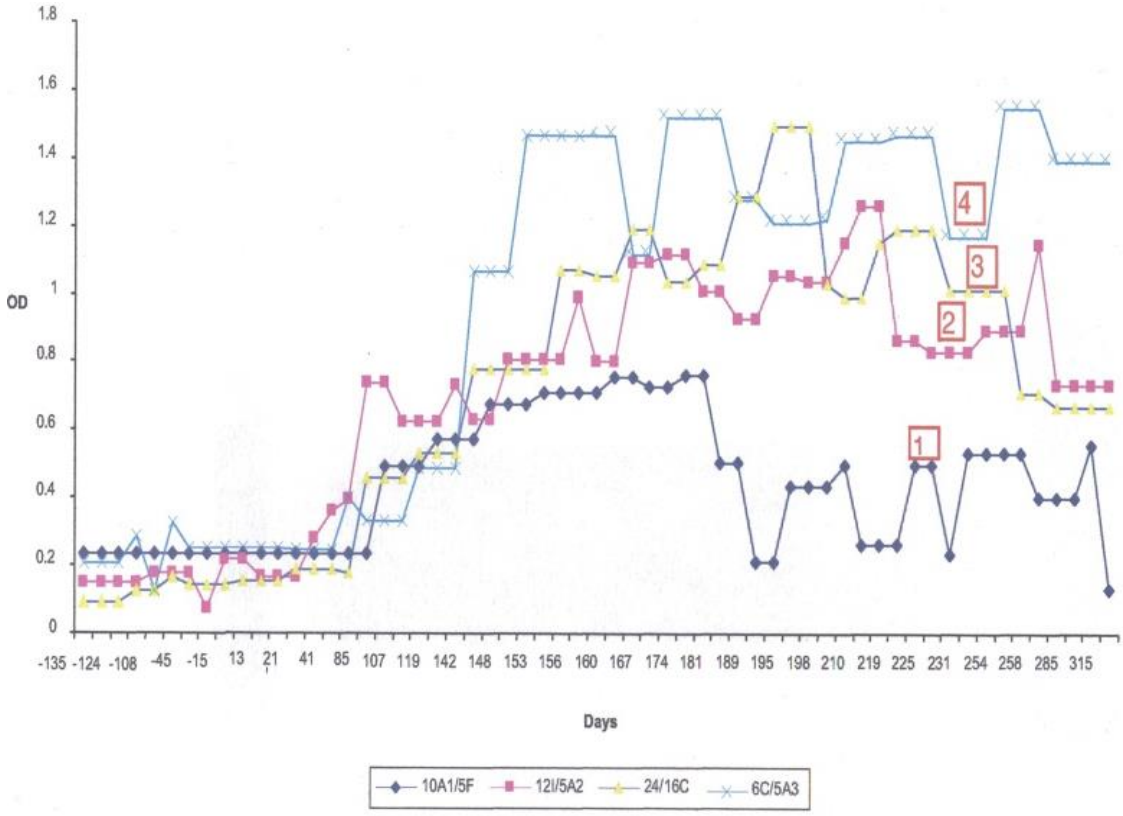

Figure 1. Kinetics of specific anti-microfilarial IgG according to L3 dose. Microfilaria-specific IgG were detected by ELISA in mandrill sera at different time points before, during and after infection. The level of specific IgG expressed as an optical density unit (OD) was plotted against the bleeding time point of mandrills. The mean level OD for each time point for a pair of animals that received the same dose was drawn. Curve 1, animals with 1000L3 ×1 dose (10A1/5F); curve 2, animals with 250L3 ×4 dose (12I/5A2); curve 3, animals with 100L3 ×1 dose (24/16C); curve 4, animals with 25L3 ×4 dose (6C/5A3).

parasitological, hematological and immunological parameters which is impracticable in natural transmission conditions in humans. In the present study, peripheral white blood cells increased with the administration of some doses (250L3 ×4), whereas with other doses only some cell types were affected. Therefore, the number of monocytes is reduced during prepatent and patency periods when either a low dose is repeated (25L3 ×4) or a single dose (100L3×1) is administered, suggesting that developing worms and mf may act on this cell type. It has been suggested that microfilaria may down-modulate monocyte function and responsiveness via antigen-presenting cells (APCs), resulting in the suppression of a Th1-type response (Sasisekhar et al., 2005). Whether this is the case here requires further study. Moreover, the state of energy characterized by low peripheral blood mononuclear cell proliferation but regular synthesis of antibodies has been described in filarial infection (Nutman et al., 1987). This phenomenon helps parasites to survive in this environment. In other cases, neutrophils are decreased during the prepatent state (25L3 ×4), whereas they increase with the appearance of mf (250L3 ×4) during patency. No noticeable change was seen with the lymphocytes. Neutrophils were shown to be one of the major cells involved in antibody-dependent cell-mediated cytotoxicity (ADCC) for *L. loa* mf (Pinder et al., 1992);

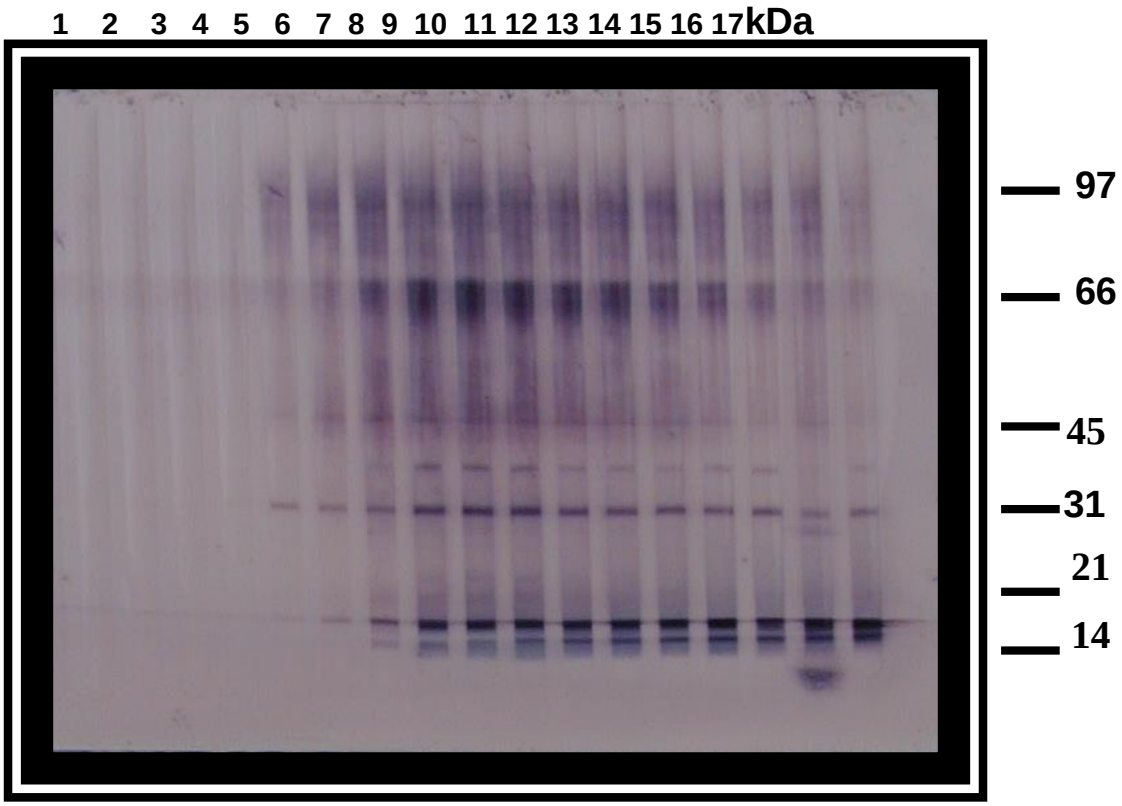

Figure 2. Microfilaria antigen recognition by mandrills during infection patency. Microfilaria antigens were separated in a SDS-Page 12.5% gel then transfer on NC paper follow by incubation with mandrill sera diluted 1/800. The reaction was then detected by anti-human IgG coupled to alkaline phosphatase diluted 1/1000. Lines 1 to 4 represent mandrill sera before infection (days 131; 108, 62, 4, respectively). Lines 5–17: mandrill sera after inoculation of L3 day 25, 61, 89, 145, 154, 160, 166, 173, 180, 194, 208, 233, 254, respectively). The number on the right shows the standard molecular weight in kilodaltons. The number on top corresponds to the different serum samples taken at different time points during the follow-up.

whether their increase during the prepatency period is related to this fact is not known.

Eosinophils present several fluctuations in many cases. While low doses (25×4; 100×1) induce a progressive rise in the quantity of eosinophils from prepatency to patency, a massive single dose (1000 ×1) did not present the same trend. It was also noted that a high sequential dose (250×4) produces more eosinophils than a low sequential dose (25×4) during prepatency. It has been shown that eosinophilia is hallmark in nematode infection. In some cases, it may play a protective role, in others, it may act as an immunoregulator or inducer of pathology (Cadman et al., 2014). In loiasis, eosinophilia has been shown but eosinophils were not a specific marker for *L. loa* infection.

However, it was shown that eosinophils are one of the cells implicated in ADCC against *L. loa* microfilaria (Pinder et al., 1992). It is likely that this variation of cells reflected the variability in action of different doses of L3 on immune cells, which will determine the early immune response and in turn may determine the outcome of infection. This hypothesis is substantiated by the fact that previous studies showed that an early immune response may affect the entire immune response (Babayan et al., 1995).

In addition, in loiasis, the cellular immune response has shown that IL5 and IL2 are elevated in these animals (Leroy et al., 1997), and the importance of these cytokines (IL5, for example) on the activity of these cells is well known (Limaye et al., 1990). This spectrum may be comparable with what is seen in humans naturally infected with multiple inoculations at different times and shows the complexity of the analysis in natural infection of humans where neither the dose nor the frequency of inoculates is known.

Although, not significant, it was apparent that the dose and regimen had an effect on microfilaremia, the animals receiving a massive single dose had the highest mf density as compared to those receiving low and repeated doses. In contrast to the reduction of the mf level obtained in an experiment using irradiated *L. loa* L3 (Akue et al., 2001), no significant reduction was seen despite the difference in doses and regimens, in this study

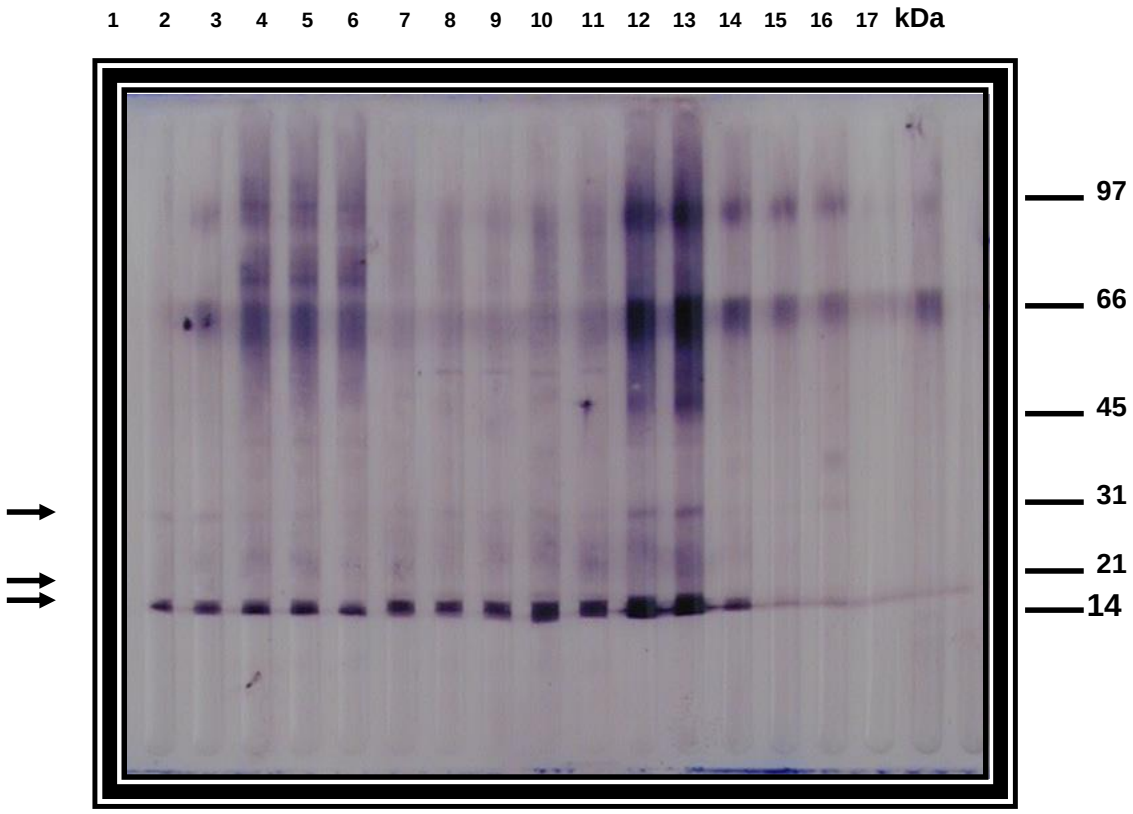

Figure 3. Kinetics of microfilarial antigen recognition in infected mandrills. Western blotting was performed after separation of microfilaria antigen in a SDS PAGE gel. Sera from mandrills diluted at 1/800 were used as probes and the reaction was revealed with anti-human IgG conjugated to alkaline phosphatase diluted at 1/1000. Lines 1–5: reactivity of mandrill with high microfilaraemia (mandrill 2A: 4–9).Lines 6–10: mandrill with low microfilaraemia (mandrill 19). Lines 11–13: mandrill with very low microfilaria (mandrill 20). End of infection: lines 14–15 (mandrill 20). Lines 16–17: noninfected mandrills. Standard molecular weight is indicated at the right. The number on the top represents different samples at different time points. Arrows on the left indicate the positions of mf 30, 20 and 14 kDa antigens.

using natural infective L3. This suggests that the mechanism involved in irradiated L3 is not the same as that in normal L3. Antibodies have been implicated in several effector mechanisms involved in killing mf. Interestingly, in this study, the animals receiving low and repeated doses presented high and persistent levels of specific anti-mf IgG, whereas a large single dose had the lowest antibody level, which tended to decrease drastically earlier than the other groups. The fact that this persistent high level with repeated low doses was not followed by a drastic drop in mf, as seen in irradiated larvae, suggesting that there is a different mechanism using either another isotype of antibodies or a different IgG subclass but this was not studied here.

Mf antigen recognition patterns were also examined. While animals without any sign of patent infection recognize mostly antigens whose molecular weight is 30 kDa or higher, animals with patent infection recognize antigens from 14 to more than 100 kDa. The recognition of some of these antigens seems not to be related to mf density like the 20-kDa antigen. The recognition of this antigen does not depend on dose. Antibodies against this antigen disappear with the end of infection. As in animals, follow-up for 4 years where mf disappeared for 6 months showed that antibodies against this antigen are no longer found in serum. This suggests that these antigens, which resemble those that have been observed in previous experiments in terms of molecular size (Akue et al., 2001), are probably a good marker of patent infection in loiasis and need further characterization. Several mandrills have been infected since 1988 to address several questions on the receptivity of this primate to the human *L. loa* and its immune response, but none has addressed the issue of dose-response outcome. Using the large serum bank and data generated, how dose can affect outcome was analyzed.

Experiments in primates are logistically difficult; therefore, any data contribution that can avoid duplications is useful to the scientific community. Since primates are phylogenetically related to humans, their use for vaccines, drugs, or diagnostic trials may be helpful.

Conclusion

The dose and regimen of natural L3 in primate were analyzed and it was found that dose and regimen may affect several parameters of the host. However, the antigen recognition remains identical from the patency to the end of infection.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was funded by the International Center for Medical Research of Franceville (CIRMF). CIRMF is sponsored by the State of Gabon, Total Gabon and the French Ministry of Foreign Affairs.

REFERENCES

Akue JP, Dubreuil G, Moukana H (2001). The relationship between parasitological status and humoral responses to *Loa loa* antigens in *Mandrillus sphinx* model after immunization with irradiated L 3 and infection with normal L3. Parasitology 123:71-76.

Antonori S, Schifanella L, Million M, Galimberti L, Ferraris L, Mandia L, Trabucchi G, Cacioppo V, Monaco G, Tosoni A, Brouqui P, Gismondo MR, Giuliani G, Corbellino M (2012). Imported *Loa loa*filariasis: Three cases and a review of cases reported in non-endemic countries in the past 25 years. Int. J. Infect. Dis.16:e649-e662.

Babayan S, Attout T, Specht S, Hoerauf A, Renia L, Korenaga M, Bain O, Martin C (2005). Increased early local immune responses and altered worm development in high dose infections of mice susceptible to the filarial *Litomosoides sigmondontis*. Med. Microbiol. Immunol. 94:151-162.

Bradford M (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. Anal. Biochem. 72:248-54.

Cadman ET, Thysse KA, Bearder S, Cheung AY, Johnston AC, Lee JJ, Lawrence RA (2014). Eosinophils are important for protection, immunoregulation and pathology during infection with nematode microfilariae. PLoS Pathog. 10(3):e1003988.

Carme B, Boulesteix J, Boutes H, Puruehnce MF (1991). Five cases of encephalitis during treatment of loiasis with diethylcarbamazine Am. J. Trop. Med. Hyg. 44:684-690.

Dennis VA, Lowrie RC Jr, Osae-Addo G, Blanchard JL (1993). Microfilarial densities, Hematologic Changes, and Serum Antibody levels in *Loa loa*-Infected Rhesus Monkeys (*Macacamulatta*). Am. J. Trop. Med. Hyg. 49:763-771.

Dupont A, Zue-N'dong J, Pinder M (1988). Common occurrence of amicrofilaraemic *Loa loa*filariasis within the endemic region. T. R. Soc. Trop. Med. Hyg. 82:730.

Gardon J, Kamgno J, Fobi G, Essiené A, Ntep M, Gaxotte P, Boussinesq M, Kollo B (1999). Depistage, identification et prise en charge des effets secondaires graves imputables à la loase et au traitement par ivermectine au cours des campagnes de lutte contre l'onchocercose. Bull. Liaison Documentation de l'OCEAC 32:137-52.

Laemmli UK (1970). Cleavage of structural proteins during the assembling of the head of bacteriophage T4. Nature 227:680-685.

Leroy E, Baize S, Wahl G, Egwang TG, Georges AJ (1997). Experimental infection of a nonhuman primate with *Loa loa* induces transient strong immune activation followed by peripheral unresponsiveness of Helper T cells. Infect. Immunol. 65:1876-1882.

Limaye AP, Abrams JS, Silver JE, Ottesen EA, Nutman TB (1990). Regulation of parasite-induced eosinophilia: selectively increased interleukine -5 production in helminth-infected patients. J. Exp. Med. 172:399-402.

Nutman B, Thomas V, Kumaraswami V, Ottesen EA (1987). Parasite-specific Anergy in Human Filariasis.Insights after analysis of parasite antigen-driven lymphokine production. J. Clin. Investig. 79:1516-1523.

Pinder M, Everaere S, Roelants GE (1994). *Loa loa*: Immunologial responses during experimental infections in Mandrills (*Mandrillus sphinx*). Exp. Parasitol. 79:126-136.

Sasisekhar B, Aparna M, Augustin DJ, Kaliraj P, Kar SK, Nutman TB, Narayanan RB (2005). Diminished monocyte function in microfilaremic patients with lymphatic filariasis and its relationship to altered lymphoproliferative responses. Infect. Immunol. 73:3385-3393.

Towbin HZ, Staehelin T, Gordon J (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA. 76:4350-4354.

Van Hoegaerden M, Ivanoff B (1986). A rapid, simple method for isolation of viable microfilariae. Am. J. Trop. Med. Hyg. 35:148-151.

Wahl G, Moukagni R, Touré F, Georges AJ (1995). Large scale collection of viable larvae of *loa loa*. Trop. Med. Parasitol. 46:203-204.