

IMMUNOEPIDEMIOLOGY OF *DRACUNCULUS MEDINENSIS* INFECTIONS I. ANTIBODY RESPONSES IN RELATION TO INFECTION STATUS

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Abstract. The specific serum IgG1, IgG4, and IgE responses to *Dracunculus medinensis* and the level of total IgE of individuals living in a highly endemic area of northern Ghana were measured by ELISA. Sera were obtained in the high transmission season from individuals with prepatent, patent, or postpatent infection as well as from individuals from the same endemic area who claimed to have never had a patent infection (i.e., endemic normal individuals). Individuals with prepatent or postpatent infections responded with a significantly lower mean level of specific IgG1 and IgG4 compared with individuals with a patent infection, and with a significantly higher mean level of specific IgG1 and IgG4 compared with endemic normal individuals. For specific IgE, no differences were found in the mean antibody level between the infection status categories. Individuals with a patent infection had a significantly lower mean serum level of total IgE compared with prepatent, postpatent, and endemic normal individuals. Endemic normal individuals had the highest mean level of total IgE. Furthermore, in all clinical categories, high responders for specific IgG1 and IgG4 generally had low levels of total IgE, whereas low responders for specific IgG1 and IgG4 generally had high levels of total IgE. A similar dichotomy, although less distinct, was observed between specific IgG1 and IgG4 on the one hand and specific IgE on the other. Thus, similar to what has been suggested for schistosomiasis and lymphatic filariasis, the relationship between the IgG subclasses and IgE appears to play a role in, or at least to reflect, a mechanism for protective immunity in dracunculiasis.

Human infections with *Dracunculus medinensis* (guinea worm) occur in parts of West and Central Africa and in Yemen and India.¹ The 10–12-month prepatent period is asymptomatic except for the last 1–2 weeks during which the adult female worm reaches subcutis and prepares for penetration of the body surface. At this late stage, the worm becomes palpable in the skin and a blister develops around its anterior end, thus making the patient aware of the infection.^{2,3} Currently, no reliable method exists for diagnosis of dracunculiasis in the asymptomatic part of the prepatent period.

Dracunculus medinensis induce vigorous humoral immune responses in infected individuals.^{4–7} However, little is known about the relationship between the humoral responses and infection status (i.e., prepatent, patent, postpatent and those claiming to have never had a patent infection). Briefly, previous studies indicated that individuals in a prepatent and patent stage of infection responded similarly with total specific immunoglobulins,⁴ and individuals in a patent and postpatent stage responded similarly with total specific immunoglobulins and specific IgG1, IgG4, and IgE.⁵ Sera from endemic normal individuals (claiming to have never had a patent *D. medinensis* infection despite living in a highly endemic area) responded only weakly with specific IgG1 and IgG4 compared with individuals with patent or postpatent infections.^{5,6}

This study elaborates on previous studies by analyzing levels of specific IgG1, IgG4, and IgE, as well as the concentration of total IgE in sera from individuals whose parasitologic status in relation to the prepatent, patent, and postpatent stages of infection were carefully determined. The postpatent category was further divided into four subcategories differing with respect to the time elapsed since the previous period of patency. The specific antibody responses against two *D. medinensis* antigen preparations, one made from adult female worms and the other made from first-stage larvae, were assessed. By comparing the antibody responses in individuals in the various infection status categories oc-

curing in endemic areas, the study provides new information about the interaction between *D. medinensis* and its human host.

SUBJECTS AND METHODS

Study individuals and study design. One hundred seventy-two individuals were selected from two communities in northern Ghana that are highly endemic for *D. medinensis* infection. Venous blood samples were collected at the onset of the study in the high transmission season in June 1991. The individuals were subsequently followed closely for 15 months (until August 1992) during which period their *D. medinensis* infection status was recorded, and repeated questioning about previous experiences with dracunculiasis was performed. Based on these data the study individuals were divided into seven clinical categories (Table 1): those who did not have a patent infection at the onset of the study but who developed one or more patent infections during the first 12 months of the follow-up period (category a: prepatent); those who had a patent infection at the onset of the study and who either did or did not develop another patent infection during the follow-up period; (category b: patent); and those who did not have a patent infection at the onset of the study and who did not develop a patent infection during the follow-up period, but who had had at least one patent infection within the last six months prior to onset of the study (category c: postpatent, 1991); during 1990 (category d: postpatent, 1990); during 1986 to 1989 (category e: postpatent, 1986–1989) or before 1986 (category f: postpatent, before 1986), or who claimed to have never had a patent *D. medinensis* infection despite living in the same endemic environment as the other villagers (category g: nonpatent, endemic normal). Five Danish individuals, who had never been to tropical countries, served as nonendemic controls (category h: nonpatent, nonendemic control). The categories were reasonably age- and sex-matched (Table 1).

The study was reviewed and approved by the Ministry of

TABLE 1
Characteristics of study individuals

Infection status category	Number (males/females)	Mean age in years (range)
a. Prepatent	17 (10/7)	33 (22–65)
b. Patent	47 (24/23)	30 (8–51)
c. Postpatent, 1991*	18 (8/10)	30 (15–55)
d. Postpatent, 1990*	17 (6/11)	31 (16–52)
e. Postpatent, 1986–1989*	21 (13/8)	34 (16–53)
f. Postpatent, before 1986*	27 (13/14)	39 (18–65)
g. Nonpatent, endemic normal	25 (14/11)	30 (15–48)
h. Nonpatent, nonendemic control	5 (3/2)	26 (20–33)
Total	177 (91/86)	32 (8–65)

* Indicates which year or period the individuals of the category experienced their last patent infection.

Health in Ghana and by the Research Council at the Danish Bilharziasis Laboratory. Informed oral consent to participate in the study was obtained from all involved volunteers.

Skin snip and night blood examinations for microfilariae indicated that the study individuals were negative for onchocerciasis and lymphatic filariasis, respectively. Examination of stool and urine for helminth infections indicated that infections with hookworm (75%) and *Strongyloides stercoralis* (13%) were common.

Preparation of serum and antigen. Serum was recovered from the blood samples after clotting and centrifugation, and sodium azide was added to a concentration of 15 mM prior to freezing the samples at -80°C until use. *Dracunculus medinensis* first-stage larvae and adult female worms obtained from infected individuals living in northern Ghana were the sources of antigen. Recovery of these parasite stages, and preparation of crude homogenates of adult worms (ADGW) and larvae (LVGW) was carried out as previously described.⁷

Semiquantitative measurement of *D. medinensis*-specific IgG1, IgG4, and IgE. Specific serum antibodies were measured by an indirect ELISA according to procedures previously described.^{5,7} The antigens were applied at an optimal protein concentration of 5.5 $\mu\text{g/ml}$. Optimal dilutions of sera and horseradish peroxidase (HRP)-labeled anti-human antibodies (conjugates) were determined by titration for each antibody type measured. Thus, for measurement of IgG1 and IgG4 the sera were diluted 1:5,000 and the conjugates (mouse-anti-human IgG1 or IgG4; Centraal Laboratorium van de Bloedtransfusiedienst, Amsterdam, The Netherlands) were diluted 1:2,000. For measurement of IgE the sera were preabsorbed for IgG using protein A (Kabi Pharmacia Diagnostics, Uppsala, Sweden) coupled to a sepharose gel in a Bio-Rad Spin Column (Bio-Rad, Hercules, CA). The preabsorbed sera were diluted 1:20 and the conjugate (rabbit-anti-human IgE; Dako, Glostrup, Denmark) was diluted 1:1,000. All sera were tested in triplicate and the results for each specimen were expressed as the mean absorbance value. To adjust for minor plate-to-plate variations, a positive control serum, consisting of a mixture of serum samples from *D. medinensis*-infected individuals, was included on all plates.

Quantification of total IgE. The total IgE concentrations in sera were measured by a sandwich ELISA procedure as described previously with only minor modifications.⁸ Rabbit anti-human IgE (Dako) was used at a concentration of 3.9

$\mu\text{g/ml}$ corresponding to a dilution factor of 1:2,000. After blocking with phosphate-buffered saline containing 0.1% Tween 20 and 0.5% bovine serum albumin for 1 hr at room temperature, 100 μl of the following were applied to each plate: 1) a two-fold serial dilution of a standard human IgE (total IgE control; Kabi Pharmacia Diagnostics) from 0.08 $\mu\text{l/ml}$ to 0.000625 $\mu\text{g/ml}$, and 2) test serum samples in dilutions of 1:50. Serum samples with high IgE concentrations were re-examined later at dilutions of 1:500. The HRP-conjugated rabbit anti-human IgE diluted 1:1,000 was used as detecting antiserum. The mean absorbance values obtained from the serial dilutions of the IgE control were used to generate a standard curve showing the relationship between absorbance value and IgE concentration for each plate. Based on this curve, the IgE concentrations in the test samples on the same plate were determined. All sera were tested in triplicate and the results were expressed as the mean of the three tests.

Data analysis. The ELISA optical density values were compared statistically with nonparametric tests (Mann-Whitney two sample U-test and Kruskal-Wallis one-factor analysis of variance). Probability values (*P* values) less than 0.05 were considered statistically significant.

RESULTS

Comparison of specific antibody responses to LVGW and ADGW. All sera were tested for specific IgG1, IgG4, and IgE against ADGW and LVGW, and individual responses to the two antigen preparations were compared (Figure 1). For each antibody type measured, the individual sera responded relatively similarly to ADGW and LVGW. Thus, the correlation coefficients (r^2) were 0.91 (IgG1), 0.81 (IgG4), and 0.96 (IgE) for sera from the prepatent category, 0.81 (IgG1), 0.81 (IgG4), and 0.84 (IgE) for sera from the patent category, 0.93 (IgG1), 0.78 (IgG4), and 0.94 (IgE) for sera from the postpatent category, and 0.82 (IgG1), 0.74 (IgG4), and 0.88 (IgE) for the endemic normal category.

Comparison of specific antibody responses in the different clinical categories. For each of the clinical categories, the mean levels of specific IgG1, IgG4, and IgE to ADGW and LVGW were calculated (Figure 2). No major differences were observed between responses to the two antigen preparations. For IgG1 (Figure 2A), sera from the patent category had a significantly higher mean antibody level compared with any of the other categories ($P < 0.01$ for category c and $P < 0.001$ for categories b and d–h, by Mann-Whitney test). For IgG4 (Figure 2B), sera from the patent category also had a higher mean antibody level compared with any of the other categories. This difference was statistically significant for all categories except for the prepatent category ($P < 0.05$ for category c and $P < 0.001$ for categories d–h, by Mann-Whitney test). For both IgG1 and IgG4 against ADGW and LVGW, there was a clear and significant tendency among the postpatent categories that the longer the period since the last patent infection, the lower was the mean antibody level ($P < 0.001$ for both isotypes and antigens, by one-factor analysis of variance). Furthermore, the mean levels of IgG1 and IgG4 in the nonendemic control category were very low and statistically different from any of the other clinical categories ($P < 0.001$ for all

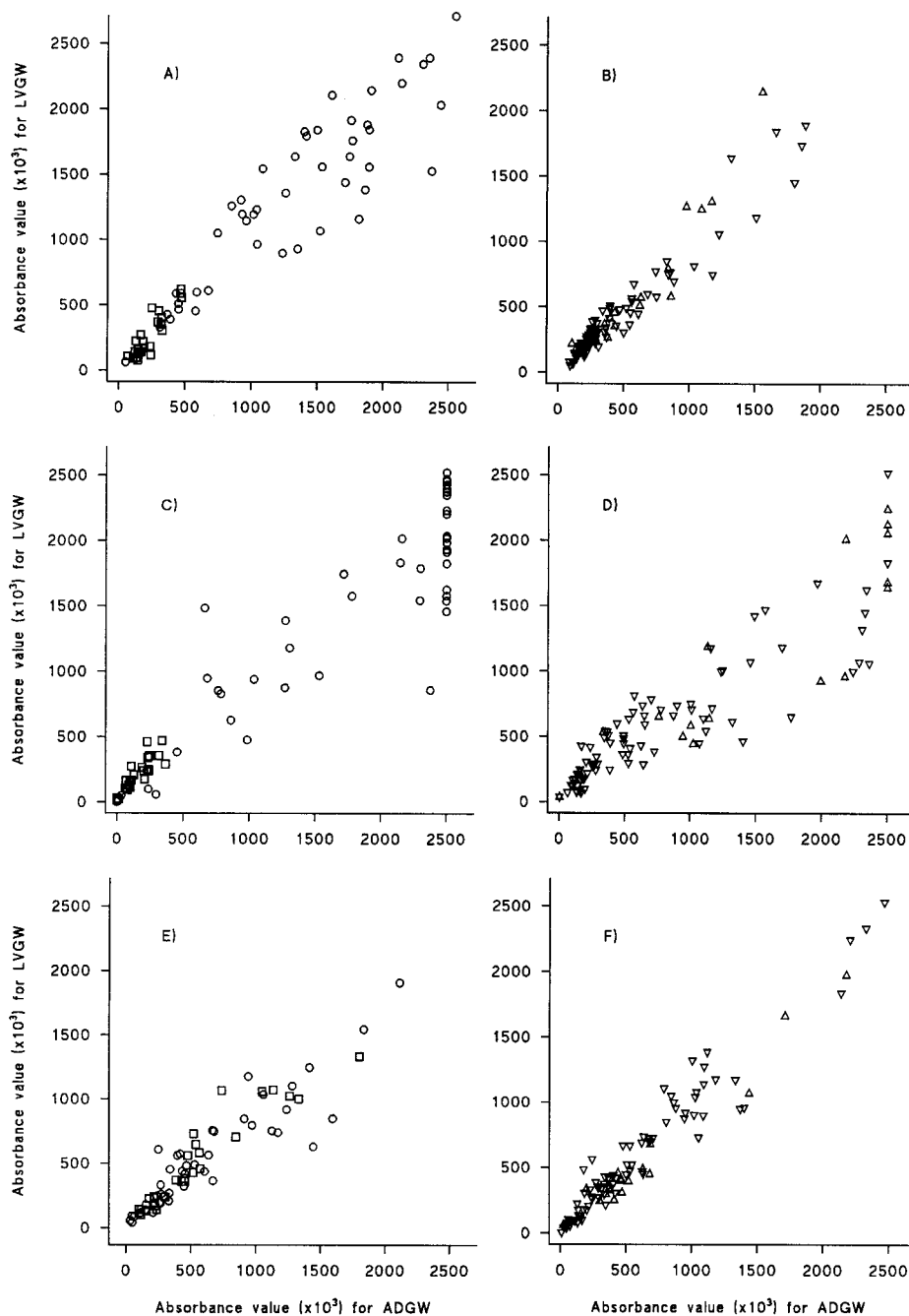


FIGURE 1. ELISA absorbance values ($\times 10^3$) for specific IgG1 (A and B), IgG4 (C and D), and IgE (E and F) antibody responses to crude homogenates of adult worms (ADGW) and crude homogenates of larvae (LVGW) for sera from individuals belonging to the prepatent category (Δ), the patent category (\circ), the postpatent category (∇) and the endemic normal category (\square). For practical reasons the four postpatent categories have been combined into one category.

comparisons, by Mann-Whitney test). No significant differences in mean absorbance values were observed between any of the clinical categories for specific IgE against ADGW or LVGW (Figure 2C), except for the nonendemic control category, which had a significantly lower mean level of specific IgE compared with any of the other categories ($P < 0.001$ for all comparisons, by Mann-Whitney test).

Concentration of total IgE in the different clinical categories. The concentration of total IgE was measured in each serum sample, and for each of the clinical categories

the mean concentration was calculated (Figure 3). Sera from the patent category had a lower mean concentration than sera from the prepatent, postpatent, and endemic normal categories. However, the differences were statistically significant only for the endemic normal category (category g; $P < 0.01$, by Mann-Whitney test) and for the two postpatent categories consisting of individuals who experienced their last patent infection in 1991 (category c; $P < 0.05$, by Mann-Whitney test) and before 1986 (category f; $P < 0.05$, by Mann-Whitney test). The endemic normal category had the highest

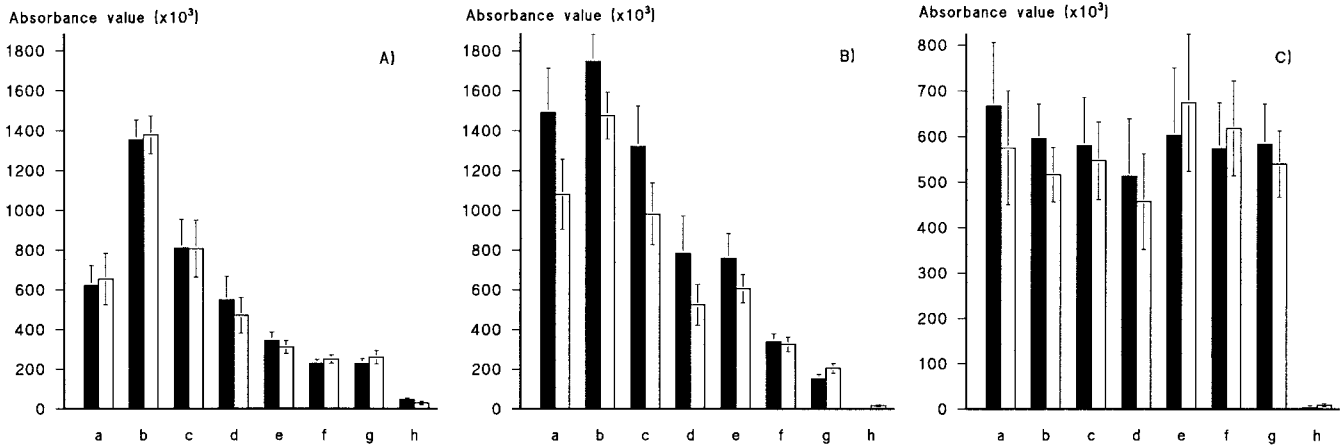


FIGURE 2. Mean ELISA absorbance values ($\times 10^3$) for specific IgG1 (A), IgG4 (B), and IgE (C) against ADGW (solid bars) and LVGW (open bars) of sera categorized as prepatent (a), patent (b), postpatent (c–f), endemic normal (g), and nonendemic control (h). Vertical bars represent standard errors of the mean. For definition of abbreviations, See Figure 1.

mean concentration of total IgE. The mean concentration of total IgE in the nonendemic control category was very low and statistically different from any of the other clinical categories ($P < 0.001$ for all comparisons, by Mann-Whitney test).

Interrelationship between the different types of antibody responses to LVGW. The interrelationship between antibody responses within the different clinical categories was analyzed by dividing individual responses arbitrarily into high and low level responses (Table 2). The percentage of individuals from each clinical category responding with low values of two antibody types compared (low-low), with

high values of two antibody types compared (high-high), and with a low value of one antibody type, and a high value of another antibody type (low-high) were estimated. For practical reasons the four postpatent categories were combined into one category. Individuals with medium response levels were not taken into consideration in this analysis.

When comparing specific IgG1 and IgG4 responses, a majority were either low level (postpatent category and endemic normal category) or high level (patent category) responders for both isotypes. Individuals from the prepatent category responded with either low or high levels of both isotypes, whereas only a few individuals (between 0% and 8.5% from each category) responded with low levels of one isotype and high levels of the other. These findings indicate that a majority of the study individuals from all four categories responded with levels of IgG1 and IgG4 that were comparable. For specific IgG1 or IgG4 responses compared with total IgE levels, between one-third and half of the study individuals from all four categories responded with low levels of one antibody type and high levels of the other. Among individuals showing this response dichotomy, those from the patent category responded exclusively with high levels of the IgG subclasses, whereas those from the endemic normal category responded exclusively with high IgE levels. When comparing specific IgE with specific IgG1 and IgG4, the level of response dichotomy was relatively high. Thus, a low/high response was observed in 15–40% of the sera depending on the category considered. Interestingly, a majority of the individuals from the patent category who responded dichotomously expressed high levels of specific IgG1 and IgG4 (and low levels of specific IgE), whereas a majority of the individuals from the endemic normal category who responded dichotomously expressed low levels of specific IgG1 and IgG4 (and high levels of specific IgE). Finally, when comparing specific and total IgE levels, most individuals had similar and generally low antibody levels.

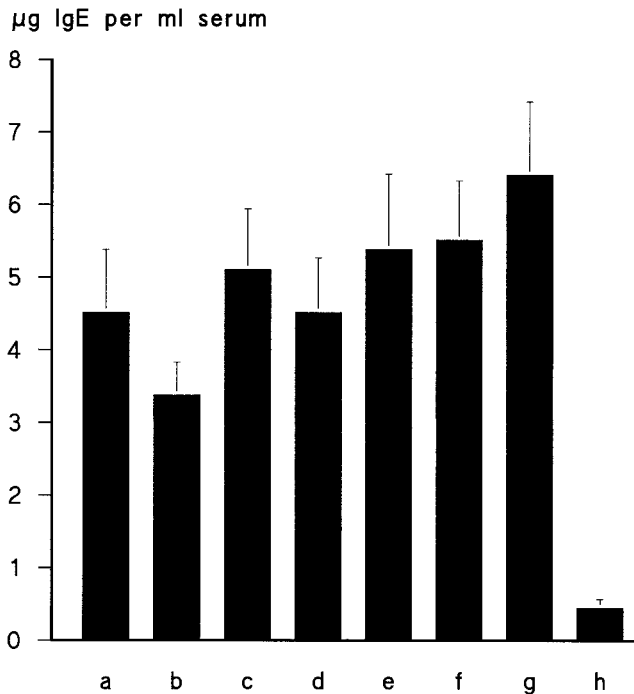


FIGURE 3. Mean concentration of total IgE of sera categorized as prepatent (a), patent (b), postpatent (c–f), endemic normal (g), and non-endemic control (h). Vertical bars represent standard errors of the mean.

DISCUSSION

This study analyzed the antibody response to *D. medinensis* infection in selected individuals from an endemic area of

TABLE 2

Interrelationship between antibody responses within each clinical category. A low level response had an absorbance value below 0.5 for specific responses and below 2.5 $\mu\text{g/ml}$ for total IgE, and a high level response had an absorbance value above 1.0 for specific responses and above 5.0 $\mu\text{g/ml}$ for total IgE. specific responses relate to LVGW only*

Antibody types compared	Level of response	Percent of sera responding			
		Prepatent category	Patent category	Postpatent category	Endemic normal category
Specific IgG1 versus Specific IgG4	Low-Low	23.5	6.4	48.2	88.0
	Low-High	5.9	8.5	8.4	0.0
	High-High	23.5	57.4	3.6	0.0
Specific IgG1 versus Total IgE	Low-Low	11.8	6.4	25.3	28.0
	Low-High	35.3	36.2	31.3	48.0
	High-High	5.9	19.1	4.8	0.0
Specific IgG4 versus Total IgE	Low-Low	5.9	14.9	18.1	28.0
	Low-High	29.4	27.7	26.5	52.0
	High-High	17.6	19.1	9.6	0.0
Specific IgE versus Total IgE	Low-Low	35.3	46.8	32.5	28.0
	Low-High	17.6	4.3	8.4	12.0
	High-High	17.6	6.4	14.5	24.0
Specific IgE versus Specific IgG1	Low-Low	35.3	12.8	45.8	48.0
	Low-High	35.3	31.9	15.7	20.0
	High-High	0.0	12.8	2.4	0.0
Specific IgE versus Specific IgG4	Low-Low	11.8	14.9	32.5	56.0
	Low-High	41.2	31.9	15.7	24.0
	High-High	5.9	12.8	4.8	0.0

* LVGW = crude homogenates of larvae.

northern Ghana. Personal histories of infection together with long-term follow-up observations of the study individuals formed the basis for categorizing the study individuals into seven infection status categories. Infections with hookworm and *S. stercoralis* infections were common in the Ghanaian study population, but immunologic cross-reactions between these infections and infections with *D. medinensis* appear to be limited.^{5,7} Extensive cross-reactions have been observed between infections with *Onchocerca volvulus* and *D. medinensis*,^{4,5,7,9} but the study area is not endemic for onchocerciasis and none of the study individuals harbored microfilariae of *O. volvulus*. Therefore, the risk of confounding effects resulting from coinfection with other helminth parasites appears to be minimal.

For specific IgG1, IgG4, and IgE, the responses to ADGW and LVGW were similar, probably due to extensive sharing of major antigenic determinants between *D. medinensis* first-stage larvae and adult female worms. Immunologic studies in our laboratory have indicated that the cuticle of adult female *D. medinensis* contains epitopes resembling human proteins (Bloch P and others, unpublished data), and that sera from individuals with onchocerciasis cross-react more extensively with ADGW than with LVGW,⁷ but apparently this did not affect the results of the present study.

In relation to the infection status categories, the responses of specific IgG1 and IgG4 to ADGW and LVGW followed the same pattern, with the highest mean levels observed for the patent category, medium mean levels observed for the prepatent and early postpatent categories, and lowest mean levels observed for the late postpatent and the endemic normal categories. This probably reflects B cell proliferation induced by epitopes of late developmental stages of *D. medinensis* females. Moreover, only a few sera (less than 10%) were high responders for one of these antibody subclasses and low responders for the other. This observation probably

reflects a similarity in antigenic determinants to which IgG1 and IgG4 react, as was also indicated by previous studies in which these isotypes reacted to the same ADGW protein bands and with approximately the same intensities in Western blots.⁵ Whereas no significant differences in the mean levels of specific IgE were observed between any of the endemic infection status categories, some variation was observed in the mean concentrations of total IgE. Thus, a relatively low mean concentration was observed in the patent category and a relatively high mean concentration was observed in the endemic normal category. The findings from the data on mean antibody levels indicate that susceptibility to *D. medinensis* infection, as represented by the patent category of study individuals, is associated with high mean levels of specific IgG1 and IgG4, whereas resistance, as represented by the endemic normal individuals, is associated with high mean concentrations of total IgE.

When comparing individual responses of specific IgG1 and IgG4 with those of total IgE a similar picture was seen, with the majority of study individuals responding with low levels of one antibody type and high levels of the other. Furthermore, most individuals with a patent infection responded with high levels of specific IgG1 and IgG4, and low concentrations of total IgE, whereas most individuals from the endemic normal category responded oppositely. The observed dichotomy between total IgE antibody levels on the one hand, and specific IgG1 and IgG4 levels on the other, thus appears to reflect a complicated interaction between IgE and the two IgG antibody subclasses in the regulation of susceptibility and resistance in *D. medinensis* infections. It has been suggested that nonspecific IgE binds the low affinity IgE receptor CD23 on eosinophils, monocytes, and B lymphocytes.¹⁰ If present in elevated levels, nonspecific IgE could negatively affect a protective immune response by blocking the binding of specific IgE antibodies to

the cells. Moreover, for Bancroftian filariasis and schistosomiasis, it has been shown that dual recognition of antigens by IgG4 and IgE is common and that the antigenic determinants can be blocked by specific IgG4 when specific IgG4 and IgE are both present.^{8,11-14} Thus, a protective immune response may be effectuated by specific IgE but regulated by nonspecific IgE and/or specific IgG4.

Individuals from the endemic normal category had high concentrations of total IgE and low levels of specific IgG4 compared with individuals from the patent category. Thus, a positive correlation existed between being endemic normal (and possibly protected from infection) and having low levels of specific IgG4 and high levels of total IgE. Based on these findings, it may be speculated that specific IgG4 antibodies contribute to blocking against a protective immune response to a higher extent than nonspecific IgE. A similar suggestion has been made for schistosomiasis.¹⁵ Thus, specific IgG4 in sera from individuals with a patent infection possibly has a blocking effect on antigenic determinants, which prevents the induction of a protective specific IgE-based immune response against infection with *D. medinensis*. This is further supported by the observation that the ratio between IgG4 and IgE against LVGW decreased from 2.86 in the patent category over 1.88 in the prepatent category and 1.09 in the postpatent category (mean value) to 0.38 in the endemic normal category. The ratios between IgG4 and IgE against ADGW and between IgG1 and IgE against LVGW and ADGW were within the same magnitude.

The relatively high concentrations of total IgE among endemic normal individuals indicates that nonspecific IgE has no immediate relevance to the blocking and protection mechanisms discussed. Its occurrence in high concentrations may rather be a side effect of mechanisms regulating the production of specific IgE or other antibody types. Furthermore, since extensive dual antigenic recognition of specific IgG1 and IgG4 has been shown to exist for dracunculiasis,⁵ a similar blocking and protective role of specific IgG1 may exist. Such a role of IgG1 has also been suggested in Bancroftian filariasis.¹³ The present study thus supports hypotheses previously presented^{10,13,15} about the significance of specific IgE in the killing of helminths and, even more interesting, about the significance of specific IgG4 (and possibly IgG1) in blocking of IgE-mediated anti-parasitic effector mechanisms.

Having paid significant attention to immunologic resistance to infection, it should be mentioned, that there may be other explanations as to why a significant proportion of people living in endemic areas never develop patent *D. medinensis* infections. One explanation is that they are perfectly susceptible to infection but simply not exposed. However, since people living in most endemic areas depend on the same very few water sources, it appears unlikely that this should be the only factor involved. Alternatively, people may be asymptotically infected, i.e., harboring worms that never emerge on the body surface. Radiographic examinations of people from endemic communities in India showed calcified worms in 29% of those who denied having a history of *D. medinensis* infection.¹⁶ The mechanism behind the inhibition of normal development of these worms in the body is unclear.

The present study also provides some guidance with re-

spect to the interpretation of antibody-based immunodiagnostic tests for dracunculiasis in which it is important to be able to distinguish between prepatent and postpatent infections. Thus, the mean responses of specific IgG1 and IgG4 in individuals with prepatent (category a) and early postpatent (category c) *D. medinensis* infections were seen to be similar, whereas individuals with patent infections responded with much higher mean levels of these antibody isotypes. Development of an immunodiagnostic test based on detection of specific IgG1 or IgG4 that can distinguish patent *D. medinensis* infections from pre- and postpatent infections may therefore be simple and realistic, whereas development of a test that can distinguish between pre- and postpatent infections appears to be more problematic. In previous attempts to develop an immunodiagnostic test for dracunculiasis we used a highly specific purified homogenate of adult female *D. medinensis*.⁷ Whether prepatent and postpatent individuals respond differently in assays based on this homogenate remains to be studied.

Acknowledgements: The study was carried out as part of an agreement between the Danish Bilharziasis Laboratory and the Ministry of Health of Ghana on the strengthening of the Guinea Worm Eradication Program in the Northern Region of Ghana. We thank the Ministry of Health of Ghana for its logistic and technical support, especially Dr. Sam Bugri, Von Asigri, Lawrence Yelifari, Albano Bayitaa, and Abdul Rahman Yakubu. We also thank Mette Lund for excellent technical assistance in the laboratory in Denmark, and Dr. Birgitte Vennervald for providing valuable technical suggestions and ideas to the study.

Financial support: This study was sponsored by the Danish Bilharziasis Laboratory and the Danish International Development Agency (Danida).

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