IMMUNOEPIDEMIOLOGY OF DRACUNCULUS MEDINENSI S INFECTIONS II. VARIATION IN ANTIBODY RESPONSES IN RELATION TO TRANSMISSION SEASON AND PATENCY

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Abstract. The serum antibody responses (specific IgG1, IgG4, and IgE, and total IgE) to Dracunculus medinensis infection in humans from a highly endemic area of northern Ghana were examined regularly by ELISA over a period of one year in cohorts of individuals who developed a patent D. medinensis infection during the study period (actively infected category), or who claimed to have never had a patent infection (endemic normal category). The results were analyzed in relation to seasonality and time of patency of infection. For individuals in the actively infected category, a clear seasonal variation in the mean levels of specific IgG1 and IgG4 was found, with the highest levels late in the dry season and early in the rainy season, when transmission is high, and the lowest levels late in the rainy season and early in the dry season. Endemic normal individuals responded with low and fluctuating levels of specific IgG1 and with low and nonfluctuating levels of specific IgG4. For specific and total IgE, no seasonal variation was observed in any of the two infection status categories. In relation to time of patency of infection (only involving the category of actively infected individuals), the mean levels of specific IgG1 and IgG4 increased from two months before patency of infection, peaked during patency, and then gradually decreased for four months until a constant level was reached. No significant fluctuations in the levels of specific and total IgE were observed in relation to time of patency. The present study thus showed extensive variation in levels of D. medinensis-specific IgG1 and IgG4 (but not IgE) over time. Seasonal variations in antibody responses may also occur in other helminth infections, especially those with seasonal transmission, and these should be taken into consideration when interpreting the results of immunologic studies.

The existence of a distinct transmission season of dracunculiasis provides an ideal opportunity to describe variations in the humoral immune responses of infected individuals in relation to seasonality, or in relation to the time of patency of infection. In a previous study from northern Ghana, it was observed that people living in a dracunculiasis endemic area had levels of Dracunculus medinensis–specific antibodies (total, IgG1, and IgG4) during the time of patency, which were significantly higher than the levels measured in the same individuals eight months later, except for a few individuals who had developed a new patent infection.1 Furthermore, in rhesus monkeys infected with D. medinensis, the levels of antibodies specific to D. medinensis first-stage larvae increased from three to seven months after infection and peaked around the period of patency of infection.2 These studies indicate that the levels of specific antibodies vary over time during the course of infection. Nothing is known about the variation in the production of antibodies over time in people claiming to have never had a patent D. medinensis infection despite living in a highly endemic area.

To examine in more detail the variation over time of the humoral immune response to D. medinensis, the present study carried out a long-term assessment of these responses in individuals from a highly endemic area of northern Ghana. Thus, the variation in the levels of specific IgG1, IgG4, and IgE and in the concentration of total IgE over time was measured in individuals with active D. medinensis infection and in individuals who had never experienced a patent D. medinensis infection. The findings were analyzed in relation to the transmission season and to the time of patency. Thus, information was obtained about the seasonal variation in the production of specific antibodies and about the levels of specific antibodies produced during prepatency, patency, and postpatency of infection. Such information may add to the understanding of the relationship between D. medinensis and its human host and it may be useful in assessing the prospects for developing an antibody-based immunodiagnostic test for dracunculiasis.

SUBJECTS AND METHODS

Study individuals and study design. A cohort of 172 individuals living in an area of northern Ghana that is highly endemic for D. medinensis infection was followed closely for 15 months (from June 1991 to August 1992). During this period, the infection status of each individual was recorded, and the history of infection with D. medinensis was obtained by repeatedly interviewing each individual and/or his or her relatives. Venous blood samples were collected every two months. Thus, during 1991 blood samples were collected in June, August, October, and December, and during 1992 blood samples were collected in February, April, June, and August. By the end of the follow-up period the cohort was divided into different, clinically well-characterized categories. For the present study, two categories were selected. The first consisted of individuals who either did or did not have a patent D. medinensis infection at the onset of the study, but who developed at least one patent infection during the 15 months of follow-up (active infection category). The second category consisted of individuals who claimed to have never had a patent D. medinensis infection despite living in the same endemic environment as the other villagers (endemic normal category). From each category, 18 individuals from whom no more than one blood sample was missed during the 15 months of sampling, were randomly selected as study individuals. The categories were reasonably age and sex matched. Thus, the active infection category consisted of 11 males and seven females with a mean age of 32 years (range = 22–50 years), and the endemic normal category.
consisted of 10 males and eight females with a mean age of 31 years (range = 15–48 years).

The study was reviewed and approved by the Ministry of 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 selected study individuals were negative for onchocerciasis and lymphatic filariasis, respectively. Examination of stool and urine for helminth infections indicated that infections with hookworm (69%) and Strongyloides stercoralis (8%) were common.

**Serology.** 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 of the samples at −80°C until use. First-stage *D. medinensis* larvae obtained from infected individuals living in northern Ghana were the sources of antigen. Recovery of these larvae and preparation of a crude larval homogenate (LVGW) was carried out as described previously.

Specific serum antibodies were measured by an indirect ELISA according to procedures described previously. For measurement of specific IgE, the IgG fraction of each human serum was removed by preabsorption to Protein A prior to analysis. The LVGW antigen was applied at an optimal protein concentration of 5.5 μg/ml. Optimal dilutions of primary antibody (human sera) and secondary antibody (horseradish peroxidase [HRP]-labeled anti-human antibodies) were determined by titration for each antibody type measured. Human sera were diluted 1:5,000 for specific IgG1 and IgG4 measurements and 1:20 for specific IgE. Secondary antibody was diluted 1:200 (mouse-anti-human IgG1 and IgG4; Dako, Glostrup, Denmark). All sera were tested in triplicate and preparation of a crude larval homogenate (LVGW) was carried out as described previously.

The serum concentration of total IgE was measured by a sandwich ELISA according to procedures described previously. Human sera were optimally diluted 1:50 or 1:500 and capture antiserum (rabbit-anti-human IgE; Dako) and detection antiserum (HRP-labeled rabbit-anti-human IgE; Dako) were optimally diluted 1:1,000.

**Data analysis.** The ELISA optical density values were compared statistically with the Mann-Whitney two sample U-test for unrelated samples and the Wilcoxon matched pairs signed-rank test for related samples. Probability values (P values) less than 0.05 were considered statistically significant.

**RESULTS**

**Seasonal variation in mean antibody responses.** The mean levels of specific IgG1, IgG4, and IgE and the mean concentrations of total IgE over the study period for the two categories of study individuals are shown in Figure 1.

The mean levels of specific IgG1 (Figure 1A) were significantly higher for sera from actively infected compared with endemic normal individuals for each month of sampling (P < 0.001 for June 1991, August 1991, October 1991, and June 1992; P < 0.01 for the remaining time points). Although most distinct for actively infected individuals, a seasonal variation in the mean IgG1 levels was observed for sera of both categories, and the differences between the highest levels seen in June 1991 and April 1992 for actively infected individuals and in June 1991 and December 1991 for endemic normal individuals and the lowest levels seen in October 1991 and August 1992 for actively infected individuals and in October 1991 and June 1992 for endemic normal individuals were statistically significant (P < 0.01 for all time points).

The mean levels of specific IgG4 (Figure 1B) were significantly higher for sera from actively infected individuals compared with endemic normal individuals for each month of sampling (P < 0.001 for all time points). For actively infected individuals, the mean IgG4 levels showed a distinct seasonal variation with significant differences between the highest levels, seen in June 1991 and April 1992, and the lowest levels, seen in December 1991 and August 1992 (P < 0.01 for all time points except P < 0.05 when comparing December 1991 and April 1992). For endemic normal individuals, the mean IgG4 level decreased continuously during the study period and no seasonal fluctuation was observed. The highest level, seen in June 1991, was significantly higher than the lowest level seen in August 1992 (P < 0.001).

For specific IgE (Figure 1C), no statistical differences in mean levels were found at any time point between sera from actively infected and endemic normal individuals. Furthermore, no distinct seasonal variation was observed in the mean levels for any of the clinical categories.

The mean concentration of total IgE was higher for endemic normal individuals than for actively infected individuals at all time points of blood sampling (Figure 1D). However, these differences were not statistically significant at any time point. Despite some variation over time (with highest levels in October 1991 and lowest levels in February or April 1992), no distinct seasonal variation was observed in the mean levels for any of the clinical categories.

**Antibody responses in relation to patency of infection.** An attempt was made to analyze the antibody responses in relation to the period of patency in actively infected individuals. Thus, for each serum, the initiation month of patency of infection was set to month zero (n = 18) and the serum responses two months earlier (month −2; n = 14), four months earlier (month −4; n = 15), and six months earlier (month −6; n = 15), as well as two months later (month 2; n = 18), four months later (month 4; n = 18), and six months later (month 6; n = 11) were set accordingly. The mean levels of specific IgG1, IgG4, and IgE and the mean concentration of total IgE in relation to patency of infection are shown in Figure 2.

The mean levels of specific IgG1 (Figure 2A) and IgG4 (Figure 2B) peaked during the month of patency. Thus, for both antibody types, the levels found before patency (except at month −6 for IgG4) and at four and six months after patency were significantly lower than those found during patency (P < 0.05 for all time points before patency except P < 0.01 at month −4 for IgG1; P < 0.01 for all time points after patency except P < 0.05 at month −6 for IgG4).
mean levels of specific IgE (Figure 2C) and the mean concentrations of total IgE (Figure 2D) appeared relatively constant over time and no statistically significant differences were observed between any time points.

Variations in antibody responses in individuals. Representative examples of the individual variation in antibody responses in relation to season and period of patency are shown in Figure 3. In the active infection category individual (Figure 3A), the levels of specific IgG1 and IgG4 decreased rapidly following termination of patency in late June 1991. The levels increased again sharply prior to the new period of patency in February 1992, after which the levels again decreased. The levels of specific IgE and the concentrations of total IgE were almost constant and very low during the entire study period. For 12 of the 18 individuals from the active infection category, similar marked peaks of specific IgG1 and/or IgG4 were observed during periods of patency. Eight of these individuals also had constant low levels of specific and total IgE over time, whereas the other four had higher or fluctuating levels of specific and/or total IgE. The remaining six individuals from the active infection category had less marked peak levels of specific IgG1 and/or IgG4 during patency periods and either fluctuating or rather constant levels of specific and total IgE over time.

In the endemic normal category individual (Figure 3B), the levels of specific IgE and the concentrations of total IgE were relatively high and followed a similar course with a peak in October 1991. In contrast, the levels of specific IgG1 and IgG4 remained almost constant and very low. Of the 18 endemic normal individuals, nine responded rather similarly. For the remaining nine individuals, the levels of specific IgG1, IgG4, and IgE as well as the concentrations of total IgE were low and showed no major variation over time.

DISCUSSION

The present seroepidemiologic study analyzed in a longitudinal manner the antibody responses to *D. medinensis* infection in selected individuals living in an endemic area of northern Ghana. The only other helminths occurring to any significant extent in the study groups were hookworms and *S. stercoralis*, which do not induce notable cross-reactions with *D. medinensis* in the applied tests. Therefore,
the risk of confounding effects resulting from coinfections with other helminth parasites appeared to be minimal.

Two different aspects of the variations in antibody levels produced over time were studied. One was the variations in relation to the transmission period for dracunculiasis, and the other was the variations in relation to the time of patency of infection. Such longitudinal aspects may provide new information on the role of antibodies in *D. medinensis* infection, and on the usefulness of detection of these antibodies for diagnosis of dracunculiasis.

The mean absorbance values of specific IgG1 and IgG4 in individuals from the active infection category followed similar seasonal variations with high levels from around February to June and low levels from around August to December. In contrast, no distinct seasonal variations in specific or total IgE were observed. For individuals from the endemic normal category, a seasonal variation was observed in the mean level of specific IgG1, which followed the same pattern as observed for individuals from the active infection category, although it was much less distinct. This finding indicate that endemic normal individuals (who claim they have never had a patent *D. medinensis* infection), in reality do become infected and do respond immunologically to larval stages of the parasite. For specific IgG4, the only statistically significant difference between any of the time points in endemic normal individuals was observed when comparing the highest (June 1991) and lowest (August 1992) mean levels. This observation probably has no biological relevance since the levels were generally very low and decreased continuously over the study period. For specific and total IgE, no variations in the mean levels were observed over time in endemic normal individuals as indicated by the lack of significant differences in the mean antibody levels between any of the time points.

The period from infection with *D. medinensis* third-stage larvae until appearance of the adult female worms on the surface of the skin is 10–12 months. Studies on the transmission pattern of dracunculiasis in northern Ghana have established that peak transmission (and thereby emergence of the female worms) takes place from January to April, and that the lowest level of new cases is recorded from July to October (Bugri SZ and others, unpublished data). Thus, in the present study there was a clear correspondence between the peak transmission period and the elevated levels of specific IgG1 and IgG4 observed for the patent category (and to a lesser extent the endemic normal category) of study
individuals. This does not necessarily mean that the high levels of specific IgG1 and IgG4 are a result of transmission, i.e., of invasion by third-stage larvae, since patency of infection, i.e., the emergence of adult female worms on the body surface, occurs simultaneously. When data from the actively infected individuals were analyzed in relation to the time of patency of infection, the levels of specific IgG1 and IgG4 increased sharply (and significantly) from two months before patency of infection up to the time of patency, and then decreased gradually until six months after patency when prepatent levels were reached. For specific and total IgE, no distinct variations in the mean levels were observed in relation to the time of patency.

Whether the observed variation in antibody production is regulated by infective larvae (i.e., by transmission) and/or by adult worms (i.e., by patency) is not clear. However, positive correlations were observed between patency of infection and antibody production in a few individuals who developed patent infection beyond the peak transmission season, suggesting that antibody production is mainly induced by emerging adult worms rather than by ingested infective larvae. This issue could be further elucidated by long-term monitoring of antibody responses in a highly endemic population where transmission is effectively interrupted through provision of clean non-contaminated water by the end of a transmission season. This would eliminate the occurrence of simultaneous ingestion of infective third-stage larvae and emergence of mature female worms among the study individuals.

Significant variation in the levels and courses of individual antibody responses over time were found in both clinical categories. However, for individuals with an active infection, the majority responded with high levels of specific IgG1 and IgG4 during patency of infection and with relatively low levels of specific and total IgE. For endemic normal individuals, the majority responded with low levels of specific IgG1 and IgG4 over time and with relatively high levels of specific and total IgE. The finding that not all individuals within the same category followed the same response pattern may reflect individual differences in the levels of exposure to the parasite or in the levels of susceptibility to infection, either environmentally (i.e., related to previous experiences with dracunculiasis and/or other infections) or genetically determined.

The significance of the observed response pattern in relation to protection against development of patent *D. medinensis* infection is unclear. For dracunculiasis and other helminth infections, it has been suggested that protective immune responses in the host may be blocked by specific IgG4 (and possibly IgG1) if sharing specificity with protective specific IgE antibodies, and by nonspecific IgE if occupying Fc receptors on effector cells (especially eosinophils, monocytes, and B lymphocytes) of the immune system. It is possible that increased production of *D. medinensis*-specific IgG1 and IgG4 around the time of patency play a role in the blocking of protective immune responses, which would otherwise (e.g., in endemic normal individuals) have killed ingested infective larvae. Thus, adult female worms (or newly ingested infective larvae) may have induced these responses as a mechanism for blocking a protective response towards reinfection. This is further supported by the observation that within the peak transmission season the ratios between specific IgG1 and IgE and between specific IgG4 and IgE are much higher among the patent category of study individuals (e.g., 1.39 and 1.89 for April 1992, respectively) compared with the endemic normal individuals (e.g., 0.42 and 0.46 for April 1992, respectively).

The findings of the present study have important implications for the development of an antibody-based immunodiagnostic test for dracunculiasis that can distinguish be-

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**Figure 3.** Seasonal variation in the levels of specific IgG1 (○), IgG4 (□), and IgE (△) and in the concentration of total IgE (▲) in sera from an individual from the active infection category (A) and in an individual from the endemic normal category (B). Horizontal lines represent periods of patency of infection.
between prepatent and postpatent infections.\textsuperscript{3,5} Thus, it appears that relatively low and constant levels of specific IgG1 and IgG4 are produced from around four months after termination of patent infection until around two months before development of a new patent infection. During this period, specific IgG1 and IgG4 cannot be used as markers of infection. However, at some point during the last two months of the prepatent period an elevation in the level of specific IgG1 and IgG4 appears. Therefore, information on a patient's recent (i.e., within previous four months) experience with patent \textit{D. medinensis} infections, combined with detection of specific IgG1 and/or IgG4, may provide a means for diagnosing dracunculiasis in this last part of the prepatent period. Levels of specific and total IgE, on the other hand, appears to be of no value as a marker of infection.

The study showed extensive variation in the production of specific IgG1 and IgG4 (but not IgE) over time. The peak levels of these antibodies correlated with the peak transmission season and with the time of development of patent \textit{D. medinensis} infection. Little information is available on the seasonal variation in the antibody production for other helminth infections, but the findings of the present study may have important implications for the interpretation of results from immunologic studies on helminth infections in general, especially for those infections that are seasonally transmitted.

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