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Serum immunoglobulin and antibody levels and the passive transfer of resistance in hamsters infected with *Dipetalonema viteae*³

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Summary

Clearance of microfilariae from the circulation of hamsters infected with *Dipetalonema viteae* was demonstrated following passive transfer of serum obtained from hyperinfected hamsters. The exclusion fraction after gel filtration of this serum on Sephacryl S 200 also cleared microfilariae whereas the other fractions did not. There was no clearance with serum taken during the pre-patent, patent or latent periods of singly infected hamsters. IgM antibody to the microfilaria cuticle measured by the fluorescent antibody technique was increased 2 to 4 times in the protective serum over the other sera. Suppression of microfilaremia was also adoptively transferred by cells from infected hamsters. Serum Ig and antibody levels to microfilaria cuticle were measured in 3 strains of hamsters differing in their ability to clear microfilariae. IgM antibody to microfilaria cuticle correlated with the ability to clear. No IgG antibody to microfilaria cuticle was detected and the major Ig response to infection was in IgG₁, which increased 10 to 20 fold.

Key words: *Dipetalonema viteae*; filarioidea; hamster; immunoglobulin; antibody; passive immunity.

Introduction

Hamsters, infected with the filarial parasite *D. viteae*, can eliminate circulating microfilariae (mf) despite the continued presence of viable fecund adult

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worms (Weiss, 1970). Hamsters given a series of infections show a primary microfilaremia, gain a proportionally greater burden of fecund female worms but at no time exhibit a renewed microfilaremia (Neilson and Forrester, 1975). The mechanism of this concomitant immunity apparently operates via a T cell independent IgM response, stage specific for microfilariae (Weiss, 1978; Thompson et al., 1979). The response appears to be stimulated by microfilarial cuticular antigens, as killing and destruction of mf relies upon an opsonic effect of IgM on the mf surface assisting accessory effector cells (Weiss and Tanner, 1979). It has also been shown that serum from immune hamsters can reduce embryogenesis in vitro (Weiss, 1970) and passive immunization with immune serum can suppress the microfilaremia in vivo (Haque et al., 1978).

Not all hamster strains exhibit a transient microfilaremia; 2 inbred hamster strains sustained prolonged microfilaremia (Neilson, 1978) similar to *D. viteae* infection in jirds (Beaver et al., 1974).

In the present study we investigated the passive transfer of resistance to microfilariae with serum and/or cells within and between susceptible or resistant hamster strains, and measured qualitatively and quantitatively the immunoglobulin class and antibody levels in the serum.

Materials and methods

Animals, parasite and infections. Random bred (LVG) and inbred (LSH, CB, and PD4 strains) hamsters, 8 weeks of age were obtained from Lakeview Hamster Colony (Lakeview, NJ). Maintenance of the parasite, the infection of hamsters with infective larvae, the surgical implantation of adult worms to naive hamsters and the method for measuring microfilaremiias have been described (Neilson and Forrester, 1975; Neilson, 1979).

Sera. Fifty LVG hamsters each infected with 200 *D. viteae* larvae were bled regularly from the retroorbital sinus and the sera pooled and stored at -80°C . Sera collected between days 15–40, 65–90 and 125–150 post infection constituted the prepatent, patent and latent single-infection pools, respectively. The same hamsters were reinfected twice with 200 larvae each, between days 175–180 and between days 280–290 post primary infection. Serum collected between days 100–200 after the final infection formed the hyperinfection serum pool. Control serum was collected from uninfected age matched LVG hamsters. Prior to intraperitoneal injection, the serum pools were centrifuged at 20,000 g for 60 min at 4°C and sterilized by sequential filtration through $0.45\ \mu$ and $0.22\ \mu$ Millipore filters.

Fractionation of serum. Three ml aliquots of pooled hamster serum were gel filtered on a 26×100 cm column of Sephacryl S 200 (Pharmacia Fine Chemicals, Piscataway, NJ). The elution buffer was 0.05 Tris-HCl, pH 8.0, containing 0.14 M NaCl. Two ml fractions were eluted by ascending chromatography and flow-monitored at 280 nm. The three protein peaks eluted were pooled and concentrated on a Diaflo YM-10 ultrafilter (Amicon Corp., Lexington, MA).

Preparation of antisera to hamster Ig. Antisera to hamster Ig were prepared in rabbits following procedures described previously (Coe, 1968, 1970, 1971, 1978). The rabbit anti-hamster IgM and IgG₁ were made specific by affinity Chromatography using Cyanogen bromide-activated Sepharose 4B (Pharmacia) coupled with 17.6 mg of hamster IgG₂. Specificity of the antisera was demonstrated by immunoelectrophoresis and radial immunodiffusion.

Measurement of serum Ig and antibody. The Mancini radial immunodiffusion assay was used to measure hamster Ig and the indirect fluorescent antibody technique was used to demonstrate cuticular antibody to mf. These techniques, as well as the method used to label the antisera with

fluorescent isothiocyanate, have been described previously (Crandall and Crandall, 1971; Weiss, 1978). The concentrations of the Ig classes were expressed as percentages of the Ig in a pool of normal hamster sera. No significant differences in Ig levels were found between the hamster strains based on the measurement of 10 individual serum samples from each strain.

Cells. Uninfected or *D. viteae*-infected PD4 isogenic hamsters were killed by cervical dislocation. Spleens and the axillary, inguinal, popliteal and mesenteric lymph nodes were aseptically excised. The spleen and lymph node tissues were cut into small pieces and forced through a fine meshed stainless steel sieve into chilled Hanks' solution. The cell suspensions were washed 3 times with Hanks' solution by low speed centrifugation. Aliquots of each cell preparation were counted in a Neubauer chamber and assessed for viability by trypan blue exclusion.

Results

Serum immunoglobulin and antibody levels

Serum Ig and antibody responses were compared in 3 strains of hamsters following a subcutaneous injection of 100 larvae. Increases of over 250% in serum IgM were measured in the LVG and PD4 hamsters during patency (Fig. 1). IgM antibody to the mf cuticle was detected earlier in the LVG hamsters than in the PD4 strain, but at approximately 90 days after infection, titers were similar. The highest titers, between 1:80 to 1:60, were found at the time microfilaremia was decreasing. Serum IgM levels were consistently lower and

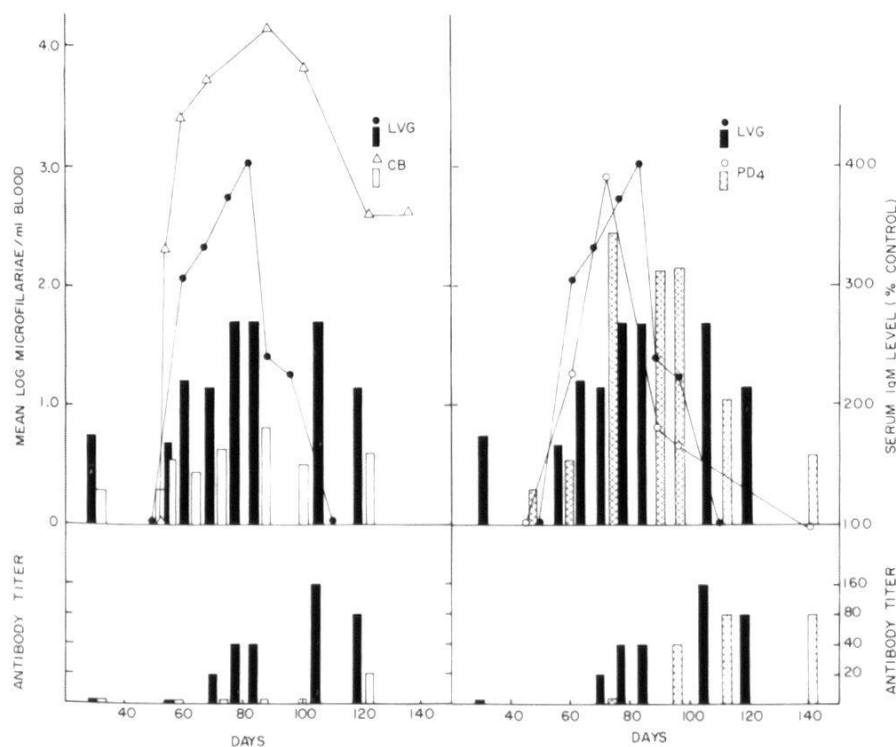


Fig. 1. Mean log microfilaria counts per ml blood, serum IgM and antibody to mf cuticle in hamsters infected with 100 *D. viteae* larvae. Each point represents the mean mf count of 7 LVG and 6 PD4 and CB hamsters except for the CB group which was reduced by mortality to 5 and 3 on days 120 and 140, respectively. IgM and antibody were measured on pooled serum samples, each animal contributing an equal serum volume to the appropriate pool.

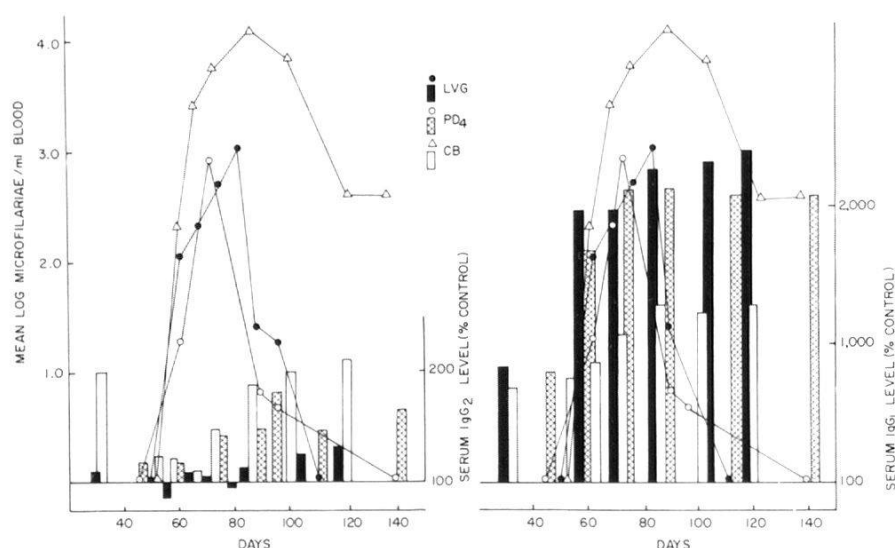


Fig. 2. Microfilaremia and serum IgG₂ and IgG₁ levels in *D. viteae* infected hamsters. See Fig. 1 for details.

microfilaremia remained high in the CB hamsters; a low titer of IgM antibody was detected only after 120 days post infection.

The IgG responses are shown in Fig. 2. Although there was an elevation in IgG₂, the major response to infection was IgG₁ with increases of over 10-fold occurring in the CB strain and 20-fold in the LVG and PD4. No cuticular IgG antibody to mf was detected.

Passive transfer of resistance with serum

The series of experiments described below were designed to (1) establish the minimum volume of a hyperinfection serum that would protect against circulating mf; (2) determine whether serum from singly infected hamsters during the prepatent, patent or latent stages, or hyperinfection serum would influence the microfilaremia resulting from infection with larvae or surgically implanted adult worms; (3) determine if the microfilaremia of a nonresponder strain would be influenced by hyperinfection serum; and (4) which Ig class of a hyperinfection serum could suppress microfilaremia.

Microfilaremic LVG hamsters infected 10 days previously by the surgical implantation of 20 adult worms of each sex, were injected intraperitoneally with 1, 2, 3 or 4 ml hyperinfection serum or 4 ml normal serum. Injections of 1 ml hyperinfection serum or 4 ml normal serum had no effect on the microfilaremi- as of recipient hamsters. Treatment with 3 or 4 ml hyperinfection serum caused complete elimination of circulating mf by day 4 post injection. No mf were observed in these animals for 12 days then mf reappeared and increased over a 14 day period to reach the control level of approximately 3.0 log mf/ml blood. After the injection of 2 ml of hyperinfection serum, a drop from 3.0 to 1.5 log mf/ml blood by day 4 was observed then the microfilaremia returned to the control level over a 6 day period.

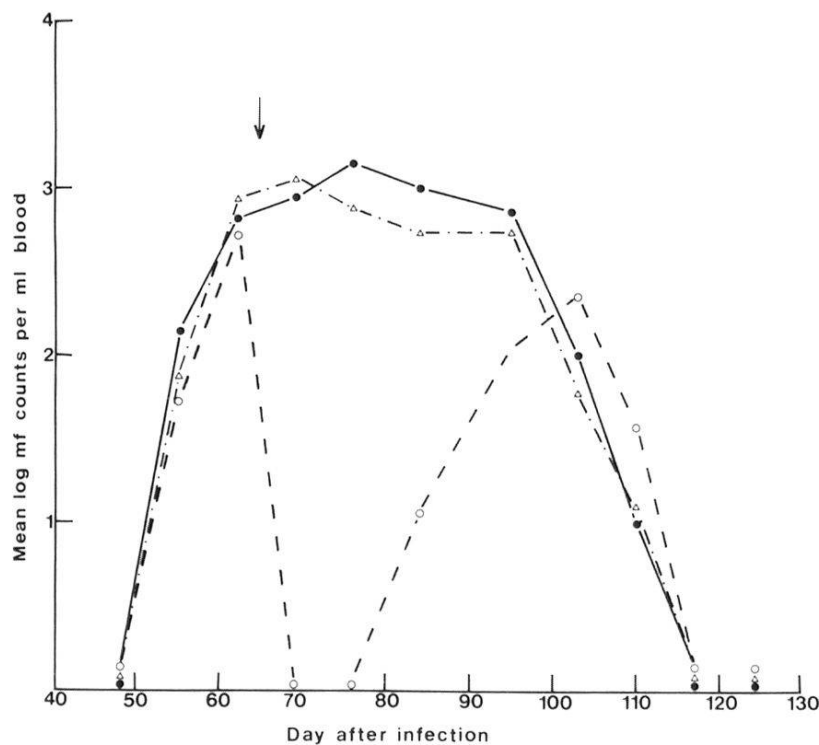


Fig. 3. Mean log microfilaria counts per ml blood of LVG hamsters (6 per group) each infected with 100 *D. viteae* larvae and injected intraperitoneally on day 65 post infection (indicated by arrow) with 3 ml saline, normal, prepatent, or patent serum (●-●); 3 ml latent serum (Δ-Δ); 3 ml hyperinfection serum (o---o).

Microfilaremic LVG hamsters infected 65 days previously with third stage larvae were injected with saline, normal serum, hyperinfection serum or serum from singly infected hamsters collected during the prepatent, patent, or latent stages. As before, 3 ml of hyperinfection serum completely suppressed microfilaremia by day 4 before returning to the control level (Fig. 3). A slight, but insignificant reduction in microfilaremia occurred after injection of latent serum while saline, prepatent, patent and normal sera injection caused no reduction. All groups of hamsters then eliminated circulating mf between days 100 and 120 post infection by the actively acquired response already described (Neilson, 1979).

No IgM antibody to mf cuticle was detected in the prepatent sera. The titer of the hyperinfection serum was 4 and 2 times greater than the patent and latent sera, respectively.

The hyperinfection serum was gel filtered on Sephacryl S 200 and 3 fractions were collected. Qualitative and quantitative details of the fractions are given in Table 1. Outbred LVG hamsters, infected by surgical implantation of adult worms and injected 2 days later with fraction 1 had a significant reduction in circulating mf levels between days 5 and 18 post transfer (Fig. 4). No reductions occurred after the injection of fractions 2 or 3. Microfilaremiias were similar in all groups of hamsters from day 22 post transfer to the end of the experiment.

Table 1. Concentration of immunoglobulin classes in hamster hyperinfection serum and fractions after gel filtration

Hyperinfection serum*	Protein conc. (mg/ml)	IgM	IgG ₁	IgG ₂
		(% normal sera)		
Unfractionated	ND**	500	3125	245
Fraction 1	6.2	240	55	< 10
Fraction 2	11.6	< 10	900	87
Fraction 3	16.7	< 10	ND**	< 10

* see materials and methods for preparation of hyperinfection serum and procedures of gel filtration

** not done

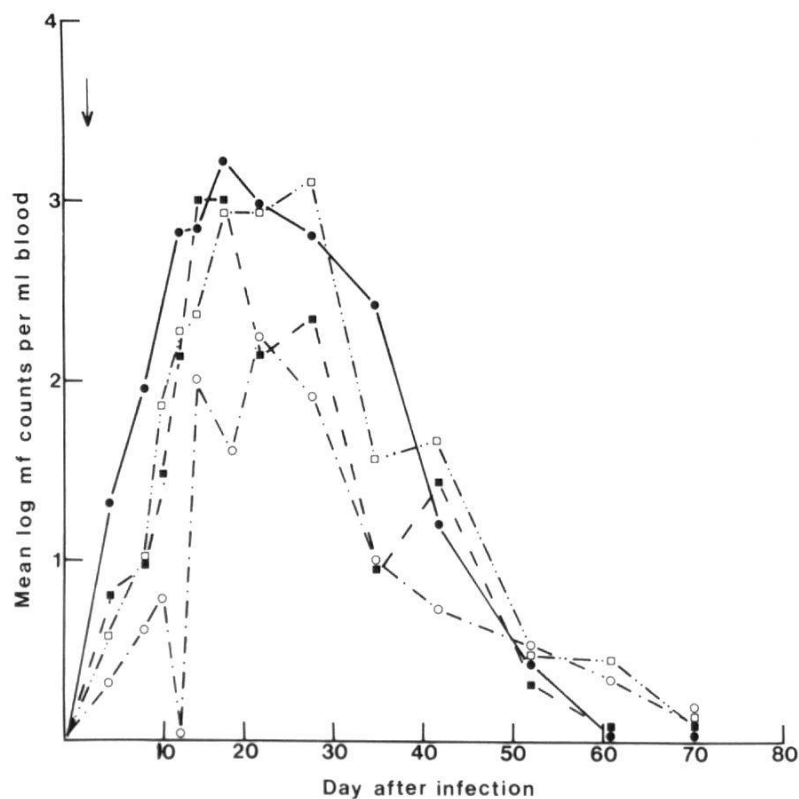


Fig. 4. Mean log microfilaria counts per ml blood of LVG hamsters (6 per group) each infected by the surgical implantation of 20 male and 20 female *D. viteae* adult worms and injected intraperitoneally on day 2 post transfer (indicated by arrow) with 3 ml whole serum equivalents of Fraction 1 (o - - o), Fraction 2 (□ - - □), Fraction 3 (■ - - ■) from hyperinfection serum separated on Sephacryl S 200, or 3 ml of gel filtration elution buffer (● - ●).

There was no apparent effect of hyperinfection, single infection or control sera on adult worm burdens of hamsters in the above experiments. Similar numbers of viable adult worms were found at necropsy regardless of serum treatment.

The LSH and CB hamster strains do not suppress or eliminate microfilariae after a primary *D. viteae* infection as do the LVG and PD4 hamster strains

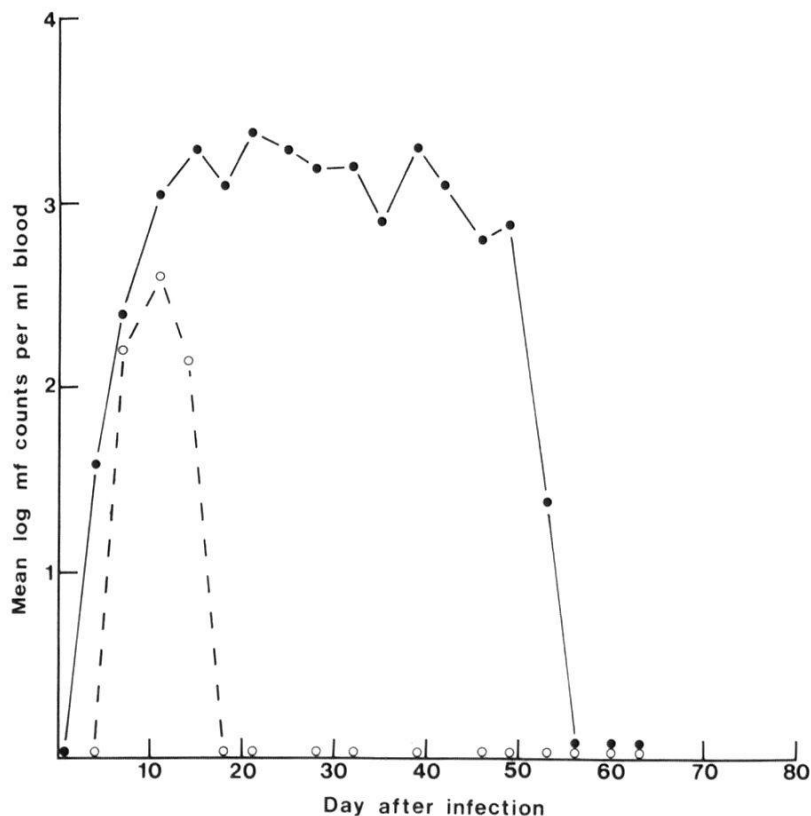


Fig. 5. Mean log microfilaria counts per ml blood of PD4 hamsters (5 per group) each infected by the surgical implantation of 20 male and 20 female *D. viteae* adult worms and injected intraperitoneally on the same day with uninfected PD4 hamster SLN cells or similar cells with normal serum (●-●); infected PD4 SLN cells (o---o); infected PD4 SLN cells with hyperinfection serum (data not plotted as the microfilaremia was zero throughout the experimental period).

(Neilson, 1978). Microfilaremic LSH hamsters were each injected with 3 ml hyperinfection serum on day 65 post infection with larvae. An immediate decline in circulating mf numbers from 3.2 log mf/ml blood to zero occurred for 10 days after which they reappeared and returned, over a 10 day period, to control levels of approximately 3.5 log mf/ml blood. This level of microfilaremia (3.5 to 4.0 log mg/ml blood) was maintained until necropsy on day 140 post infection. A similar experiment to that described above using the other nonresponder CB hamster strain gave the same results (data not shown).

Passive transfer of resistance with spleen and lymph node cells

Of the available isogenic hamster strains, only the PD4 strain suppresses circulating mf as effectively as the outbred LVG strain (Neilson, 1978) and was the strain of choice for the following cell transfer experiments. Spleen and lymph node (SLN) cell preparations were obtained from PD4 hamsters, uninfected or infected 150–200 days previously with 200 *D. viteae* larvae per hamster. Each recipient was injected intraperitoneally with the entire cell preparation from one donor.

Infected hamsters receiving normal SLN cells, normal SLN cells plus normal serum or no treatment, had similar microfilaremiias and their combined

data are plotted in Fig. 5. Suppression of circulating mf was noted on day 14 post transfer with complete elimination on day 18 in hamsters injected with immune SLN cells. Hamsters injected with immune SLN cells plus hyperinfection serum failed to develop a measurable microfilaremia during the experimental period. At necropsy all hamsters had similar adult worm burdens.

To determine when during a primary *D. viteae* infection, PD4 hamster cells were able to transfer mf clearance, SLN cells were obtained on days 20, 40, 60, 80, 100, 130 and 160 post infection with 200 *D. viteae* larvae and injected into microfilaremic PD4 hamsters. Cells harvested on days 20, 40, 60 and 80 post primary infection had no effect upon the microfilaremia in recipient hamsters. Complete suppression of circulating mf, from day 6 post transfer, occurred in hamsters receiving SLN cells from donors killed on days 100, 130, and 160 post primary infection.

Discussion

Several studies have implicated a pivotal role for antibody in the host immune mechanism responsible for the clearance of circulating mf in hamster-*D. viteae* infections (Weiss, 1970; Weiss and Tanner, 1979). In the present study the most effective suppression of circulating mf, required the inoculation of at least 3 ml of serum from hyperinfected hamsters. Lesser volumes of hyperinfection serum and 3 ml of serum from the prepatent, patent and latent stages of a primary infection either failed or only slightly reduced the microfilaremia. The transfer of insufficient antibody may well be the reason for this failure. Hyperinfection serum contained at least twice as much antibody to mf cuticle, as measured by the fluorescent antibody test, than did latent infection serum.

Only the excluded fraction 1 from gel filtered hyperinfection serum reduced microfilaremia when injected into microfilaremic hamsters. This corroborates the findings of Weiss and Tanner (1979) who demonstrated that IgM antibody was necessary for the dependent cell cytotoxic (ADCC) mechanism of mf killing. Haque et al. (1978) obtained protection with sera collected during the prepatent and latent stages of a primary *D. viteae* infection in hamsters but did not state the volume or frequency of serum injections which makes a comparison of results difficult.

The responder LVG and PD4 strains suppress circulating *D. viteae* mf within 120 days post primary infection while the non responder LSH and CB strains do not (Neilson, 1978). The longevity of adult *D. viteae* in all strains was similar. The present studies showed that the non responder LSH strain eliminated mf when given protective serum from a responder strain. Thus, the parenteral administration of antibody probably allowed the expression of the ADCC mechanism for mf destruction. This infers that the non responder hamster strains possess all components of an ADCC mechanism except antibody and that the prolonged microfilaremias noted in the non responder LSH and CB

strains may reflect an inability to produce sufficient specific IgM antibody. Additional circumstantial evidence in support of this view, comes from the observed rebound to serum preinjection mf levels, in LSH hamsters, at a time when one would predict a substantial reduction in the concentration of passively transferred antibody through catabolic processes. Although antibody was not measured in these LSH hamsters, serum IgM levels were consistently lower than in the LVG and PD4 strains during infection (unpublished).

Consistent with this hypothesis, the nonresponder CB hamster had consistently lower antibody, IgM and IgG₁ levels compared to the responder LVG and PD4 strains during a primary infection. Whether this is the result of the higher microfilaremiæ seen in CB hamster with the attendant in vivo absorption of free antibody to mf, or a basic inability of this strain to produce sufficient protective IgM antibodies remains unanswered.

What the significance of the increase in IgG₁ levels is, remains to be determined. Several other studies have shown dramatic elevations of IgG₁ in mice infected with other parasitic helminths (Crandall and Crandall, 1971; Crandall and Crandall, 1972; Crandall et al., 1974; Sher et al., 1977). Hamster IgG₁ antibody is non-complement fixing, is strongly cytophilic for hamster macrophages and has only weak phagocytosis promoting activity (Portis and Coe, 1975).

Coe (1978) in a review article described a differential induction of IgG₁ and IgG₂ in hamsters following immunization with a variety of antigens and adjuvants. A study is in progress to identify the antigen(s) in the filaria responsible for the very elevated IgG₁ responses observed during infection.

Complete and lasting suppression of circulating mf was adoptively transferred from infected to naïve PD4 hamsters. Spleen and lymph node cells from hamsters in the prepatent or early patent phases of the infection failed to confer resistance. The transfer, to microfilaremic hamsters, of SLN cells from hamsters in the late patent phase when mf levels begin to fall, or in the latent phase resulted in the complete elimination of mf. Tanner and Weiss (1979) recently showed that only B lymphocytes adoptively transferred mf clearance in isogenic LSH hamsters.

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