

# Profound suppression of cellular proliferation mediated by the secretions of nematodes

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## SUMMARY

*Loss of T lymphocyte proliferation and the emergence of a host response that is dominated by a Th2-type profile are well-established features of human filarial infection. Down-regulation and modulation of host T cell responses during lymphatic filariasis has been investigated by implantation of parasite stages into inbred mice. Adherent peritoneal exudate cells (PEC) from mice transplanted with adult or larval Brugia malayi parasites are profoundly anti-proliferative but do not prevent antigen-specific cytokine production by T cells. We demonstrate here that the excretory/secretory (E/S) products of the adult parasite are sufficient to induce PEC that block proliferation if injected daily into mice. We have previously shown that in vivo production of host IL-4 is required for the generation of suppressive cells. Because the induction of host IL-4 is characteristic of infection with nematodes, we asked whether E/S from two other nematode parasites, Nippostrongylus braziliensis and Toxocara canis were also capable of generating a suppressor cell population. Injection of E/S from these two parasites also led to a reduction in T cell proliferation suggesting that this mechanism of down-regulating host responses is a feature common to nematode parasites.*

**Keywords** *Brugia, IL-4, tolerance, T-cell, antigen presenting cells, filaria*

## INTRODUCTION

Chronicity, immune suppression and Th2-type immune responses are characteristic features of human infection with multicellular parasites but the relationship between these features remains largely unresolved. Immune suppression and Th2 responses have been attributed to the chronicity of infection with these parasites which include nematodes, trematodes and cestodes. However, long-term parasite survival is almost certainly dependent on immune suppression from the onset of infection while the importance of Th2 cytokines in mediating protection vs suppression is an area of sometimes heated debate (Allen & Maizels, 1997).

We have been particularly interested in the relationship of Th2 responses to immune suppression as well as trying to consider what common attributes of multicellular organisms might lead to profound down-regulation of host immune responses. Impaired T cell responses have been described in some detail for individuals infected with the filarial nematodes, *Brugia malayi* and *Wuchereria bancrofti* (Ottesen *et al.* 1977, Piessens *et al.* 1980, Maizels & Lawrence 1991, Yazdanbakhsh *et al.* 1993). Peripheral blood lymphocytes from people with active infection (in the absence of overt disease) do not proliferate in response to parasite antigen but produce antigen-specific IL-4. However, proliferative responses to other antigens are unimpaired.

We set out to investigate whether T cell hypo-responsiveness could be due to a defect in antigen presenting cell function caused by infection with filarial nematodes. To provide a source of *in vivo* parasite-exposed APC, we implanted mice in the peritoneal cavity with live *Brugia malayi* parasites and asked the question: Can APC from infected animals present antigen to a T cell clone (D10.G4) as effectively as cells from control animals? We found that infection-derived peritoneal exudate cells (PEC) are profoundly anti-proliferative (Allen *et al.* 1996). In the presence of these cells, T cells fail to proliferate but produce high levels of cytokine in response to cognate antigen. Antigen processing and presentation are thus fully intact. The

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proliferative block is apparently an active process that cannot be accounted for by an absence or defect in co-stimulation as T cells do not divide in response to antigen, even with the addition of excess control APC. Although these observations have striking similarities to human peripheral blood hypo-responsiveness, they differ in that the anti-proliferative effect is non-specific, blocking mitogen responses as well as preventing the proliferation of many different cell types (Allen *et al.* 1996).

During our investigations, we noted a striking correlation between the ability of certain parasite life-cycle stages to induce systemic IL-4 and the induction of profound proliferative suppression. Thus, the infective larval (L3) and adult stages, which induce high levels of parasite-specific IL-4 in the spleen, are both capable of inducing profound PEC-mediated suppression whereas microfilariae (Mf) which induce systemic IFN- $\gamma$  generate a moderate suppressive effect that can be fully reversed with inhibitors of nitric oxide (Lawrence *et al.* 1994, Allen *et al.* 1996, MacDonald *et al.* 1998). Using both neutralizing antibodies and IL-4 deficient mice, we have demonstrated that although IL-4 plays no direct role *in vitro*, the development of a suppressor cell population induced by filarial infection is critically dependent on IL-4 *in vivo* (MacDonald *et al.* 1998).

To determine the mechanism by which filarial nematodes might generate suppressor APC, we have asked whether excretory/secretory (E/S) products of *B. malayi* could duplicate the effect of implantation with live adult parasites. E/S was found to generate suppressive PEC as effectively as the live parasite itself which has significant implications for our conceptual understanding of the processes leading to suppression *in vivo*. Further, given that IL-4 is required for the generation of this suppressive mechanism (MacDonald *et al.* 1998), we asked whether E/S from two other nematode species known to be strong IL-4 inducers (Finkelman *et al.* 1988; Lawrence *et al.*, 1996); (R. Maizels, pers. comm.) could generate a similar form of suppression and found that this was indeed the case.

## MATERIALS AND METHODS

### Parasite material

*B. malayi* adults and microfilariae (Mf) were obtained from infected jirds purchased from TRS laboratories (Athens, GA, USA). Adult worms were removed from the peritoneal cavity and washed in RPMI 1640 (Gibco) supplemented with 50  $\mu\text{g/ml}$  gentamicin. Adult parasite E/S was obtained by culture of one adult worm per ml at 37°C in 50 ml RPMI supplemented with 2 mM glutamine, 100 U/ml penicillin, 100  $\mu\text{g/ml}$  streptomycin and 1% glucose. 40 ml of

supernatant was harvested daily and replaced with the same volume of fresh supplemented media. Harvested supernatant was passed through a 0.2  $\mu\text{m}$  filter and stored at -70°C prior to use. Adult *N. brasiliensis* E/S was obtained by culture of 125 worms/ml in RPMI 1640 supplemented with 2% glucose, 2 mM glutamine, 100 U/ml penicillin and 100  $\mu\text{g/ml}$  streptomycin at 37°C. Supernatants were collected daily from day 2 of culture, passed through a 0.2  $\mu\text{m}$  filter and stored at -70°C. *T. canis* larval E/S was obtained by culture of 2500 infective larvae/ml at 37°C in RPMI supplemented with 100 U/ml penicillin, 100  $\mu\text{g/ml}$  streptomycin, 2  $\mu\text{g/ml}$  Fungizone (Gibco), 15  $\mu\text{g/ml}$  gentamicin (Gibco) and 1% glucose. Supernatants were removed weekly and filtered through a 0.2  $\mu\text{m}$  filter prior to storage at -70°C. Dead parasites were collected from those that had died after overnight *in vitro* culture.

### Generation of suppressive peritoneal exudate cells

Six-to-eight-week old male CBA/Ca mice purchased from Harlan-UK (Bicester, UK) were implanted intra-peritoneally (i.p.) with either the given number of live adult *B. malayi* females, or with ten dead female adults. After three weeks mice were euthanized by cardiac puncture and PEC were harvested by thorough washing of the peritoneal cavity with 15 ml of RPMI containing 50  $\mu\text{g/ml}$  gentamicin. For investigation of the ability of parasite E/S to generate suppressive PEC, mice were injected daily for two weeks with 1 ml of *B. malayi*, *N. brasiliensis*, or *T. canis* E/S, or with RPMI alone. After this time, peritoneal cells were harvested as described above.

### Proliferation and cytokine assays

Unless otherwise stated, all *in vitro* cultures were carried out in RPMI 1640 medium (Gibco) supplemented with 2 mM glutamine, 100 U/ml penicillin, 100  $\mu\text{g/ml}$  of streptomycin, 5  $\mu\text{M}$  2-mercaptoethanol and 10% FCS (complete medium).

For investigation of the ability of parasite- or E/S-exposed PEC to present antigen *in vitro*, 100  $\mu\text{l}$  PEC at  $1 \times 10^6/\text{ml}$  were adhered to a flat-bottomed 96 well plate (Nunclon) at 37°C for 2–3 h, after which non-adherent cells and Mf were removed by washing with 100  $\mu\text{l}$  of complete medium/well. Proliferation of the conalbumin-specific Th2 cell clone D10.G4 (Kaye *et al.*, 1983) was determined in the presence of adherent PEC by adding  $5 \times 10^4$  D10.G4 cells to each well to a final volume of 200  $\mu\text{l}/\text{well}$ . Conalbumin (Sigma), the cognate antigen for the D10.G4 clone, was used at a final concentration of 50  $\mu\text{g/ml}$  where indicated. The NO inhibitor L-N<sup>G</sup>-monomethyl-arginine (L-NMMA) and the control inhibitor D-NMMA (Wellcome Foundation, Beckenham,

Kent, UK) were used where indicated at a final concentration of 250  $\mu\text{g/ml}$ . After incubation for 48 h at 37°C, 100  $\mu\text{l}$  of supernatant was removed from each well of the D10.G4 assay for cytokine analysis. After supernatant removal, 1  $\mu\text{Ci}$  [ $^3\text{H}$ ] thymidine in 10  $\mu\text{l}$  complete medium was added to each well, and plates were incubated for 16–18 h at 37°C prior to harvesting and counting using a Top Count Microplate Scintillation Counter (Canberra Packard).

The IL-2/IL-4 responsive NK cell line (Swain *et al.* 1981) was used to measure cytokine levels by D10.G4 culture supernatants, as previously described (Lawrence *et al.* 1994, Allen *et al.* 1996). In brief, proliferation of the NK cells at  $10^4$  cells/well was measured after the addition of 20  $\mu\text{l}$  culture supernatant with anti IL-2 (S4B6) neutralizing antibody. Standard curves were established using mouse recombinant IL-4 (Sigma, I-1020). Cells and supernatants were incubated for 24 hours at 37°C prior to addition of 1  $\mu\text{Ci}$  of [ $^3\text{H}$ ] thymidine in 10  $\mu\text{l}$  complete medium. After a further 12 h incubation at 37°C, plates were harvested and counted. S4B6 ascites was used at 2.5  $\mu\text{l/ml}$ , the optimal concentration for neutralization as determined by titration.

### Statistical analysis

The Student's *t*-test and paired *t*-test were used to determine the statistical significance of differences between and within groups.  $P < 0.05$  was considered to be a significant difference.

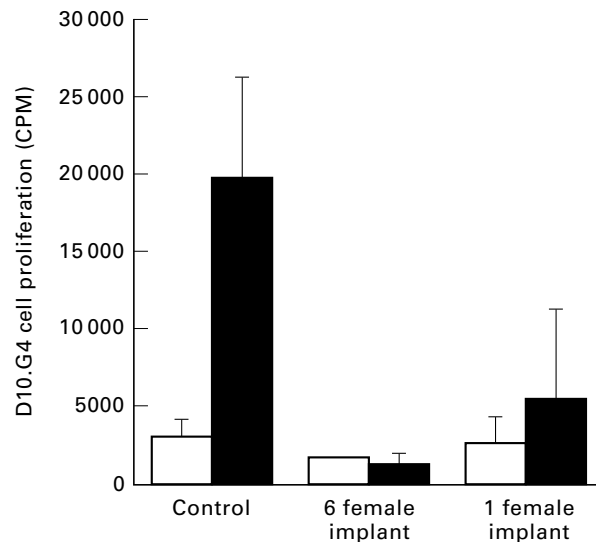
## RESULTS

### Suppression can be generated by a single adult parasite

We have previously shown that mice implanted i.p. with six *B. malayi* adult parasites generate a population of adherent peritoneal cells capable of blocking the proliferation of a range of lymphocytes (Allen *et al.* 1996). To determine the minimum number of live parasites required to generate proliferative suppression, we investigated the ability of PEC from mice implanted with a single *B. malayi* female to block proliferation of D10.G4 cells, compared to a multiple female implant. We found that significant suppression could be achieved with the implant of only one adult female parasite ( $P < 0.02$ ) (Figure 1).

### E/S induction of suppression

Having established that suppressive PEC could be generated by i.p. implant of only one adult female parasite, and given that filarial suppression of proliferation ensues from live, but not dead, adult implants (Allen *et al.* 1996), we investigated the suppressive potential of the secreted products of the

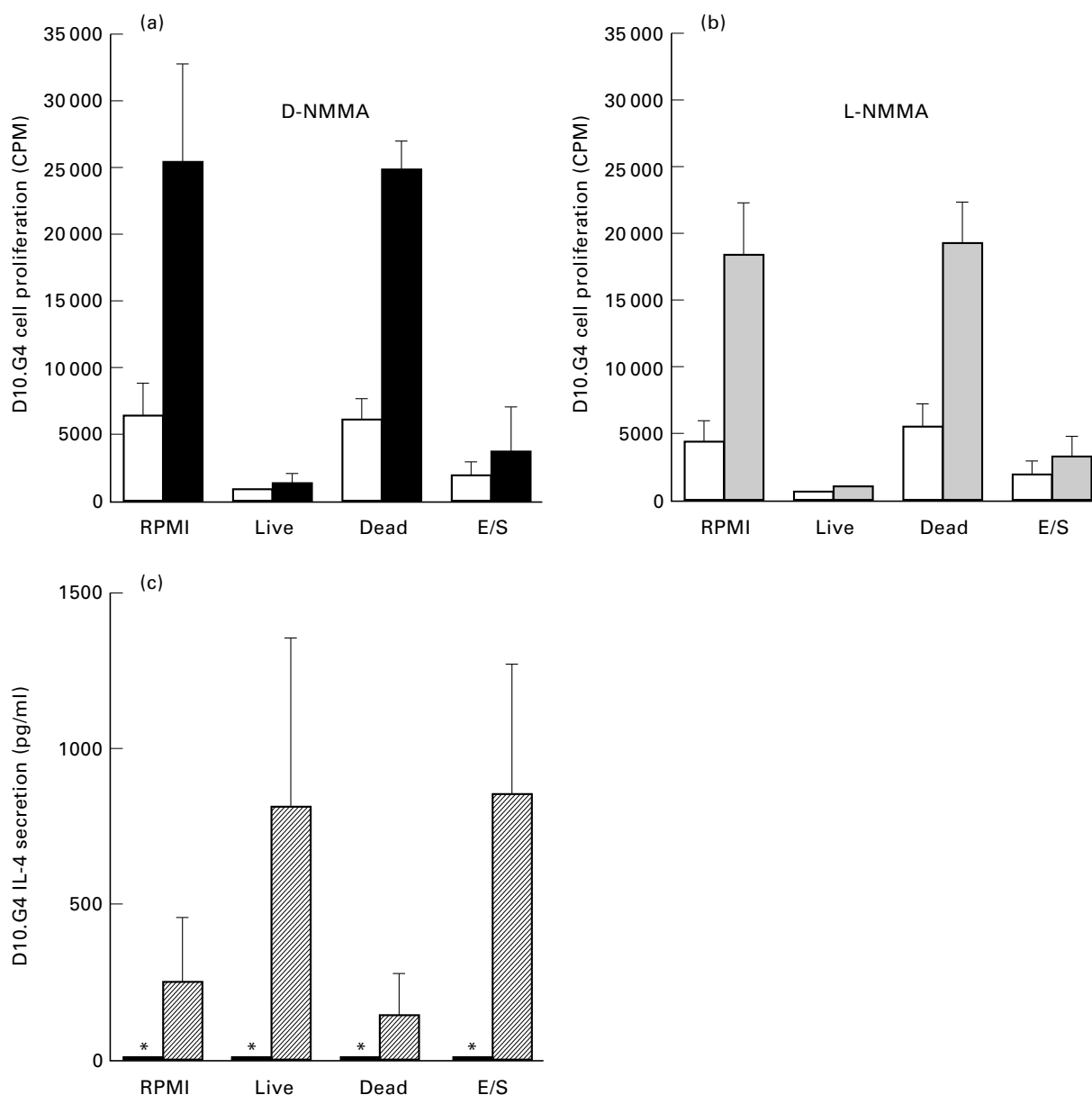


**Figure 1** Single parasite implant. Proliferation of the D10.G4 clone with media (open bars) or 50  $\mu\text{g/ml}$  conalbumin (solid bars) co-cultured with PEC from control (unimplanted), single or multiple (six) female adult *B. malayi*-implanted CBA/Ca mice. Proliferation was measured by [ $^3\text{H}$ ] thymidine incorporation and is shown as c.p.m. Data are mean  $\pm$  SD of five individual mice separately assayed.

adult stage of the parasite. We cultured *B. malayi* adults at 1 worm per ml of culture medium and collected supernatants every 24 h. Thus, administration of 1 ml/animal/day equated to the amount of E/S produced by one worm in 24 h. Daily administration of this amount of E/S i.p. for two weeks resulted in the generation of a suppressive PEC population which ablated proliferation (Figure 2a) ( $P < 0.01$ ) but not antigen-specific cytokine production (Figure 2c) in the D10.G4 system. Suppression was not significantly altered on the addition of the NO inhibitor L-NMMA (Figure 2b). Thus, administration of adult ES exactly paralleled the result with implantation of live adults parasites. Interestingly, administration of adult E/S every second day did not induce suppressive PEC (data not shown). This may reflect the low concentration of the ES used in these experiments, or may suggest that development of suppression requires constant exposure of host cells to parasite antigen. Additionally, the very effective suppression generated by adult E/S *in vivo* could not be duplicated by the direct addition of the same E/S to cultured T cells *in vitro* (data not shown).

### Suppression can be induced by E/S of several nematode species

Having established that *B. malayi* E/S has the capacity to generate suppressive PEC, we decided to test E/S from two other nematode species available in the laboratory. E/S was



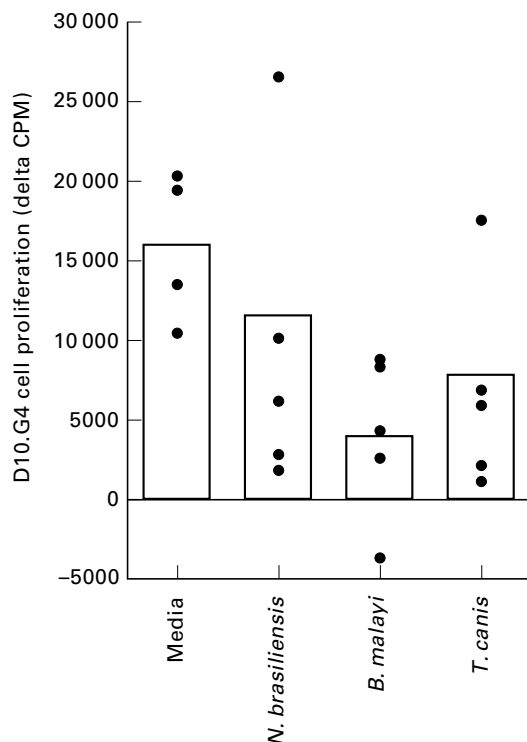
**Figure 2** Induction of suppression by *in vivo* administration of *B. malayi* E/S. Proliferation of D10.G4 cells (a) with media plus D-NMMA (open bars) or 50 µg/ml conalbumin plus D-NMMA (solid bars), or (b) with media plus L-NMMA (open bars) or 50 µg/ml conalbumin plus L-NMMA (flecked bars) co-cultured with PEC from mice implanted with; six live adult *B. malayi*, ten dead adult parasites, or injected daily with 1 ml RPMI or 1 ml adult parasite E/S. (c) D10.G4 IL-4 production in media plus D-NMMA (open bars) or 50 µg/ml conalbumin plus D-NMMA (dark hatched bars), as measured by NK bioassay. Asterisks represent cytokine production of <10 pg/ml. Proliferation was measured by [<sup>3</sup>H] thymidine incorporation and is shown as c.p.m. Data are mean ± SD of 3–4 individual mice separately assayed. Significant D10.G4 proliferative suppression was seen with daily administration of adult parasite E/S, in comparison to RPMI-injected animals ( $P < 0.01$ )

generated from adult *Nippostrongylus brasiliensis* and larval stage *Toxocara canis*. A similar biomass of parasites was used to generate E/S from these nematodes. We administered one ml of E/S to each animal *i.p.* every day for two weeks, then removed the PEC and tested their ability to stimulate antigen-specific proliferation of the D10.G4

clone. Decreased proliferation of the D10.G4 clone was seen when co-cultured with PEC from mice that had been exposed to E/S material from all three nematode species tested, although significant suppression was only achieved using PEC from *B. malayi* or *T. canis* E/S injected animals ( $P < 0.01$  and  $P < 0.05$ , respectively).

## DISCUSSION

The full developmental cycle of the human filarial parasite, *Brugia malayi*, does not occur in the laboratory mouse but individual stages of the parasite can survive for considerable lengths of time when implanted into the peritoneal cavity (Lawrence 1996). We have used this approach to study modulation of the host cytokine responses by this parasite (Lawrence *et al.* 1994, 1995) as well as the response to individual antigens (Allen *et al.* 1995). More recently, we have tried to address the impact of infection on antigen-presenting cell function as a means to understanding the dramatic down-regulation of parasite-specific T cell proliferative responses observed during infection with filarial parasites (Allen *et al.* 1996, MacDonald *et al.* 1998). We found no apparent defect in antigen-specific processing or co-stimulation but rather that peritoneal cells derived from infected animals exert a profound and non-specific anti-proliferative effect on cells with which they are co-cultured *in vitro*.



**Figure 3** E/S-induced suppression by other nematode species. Proliferation of the D10.G4 clone when co-cultured with PEC from control (unimplanted) CBA/Ca mice, or mice injected daily for two weeks with 1 ml of *N. brasiliensis*, *B. malayi*, or *T. canis* E/S. Proliferation was measured by [<sup>3</sup>H] thymidine incorporation and is shown as delta cpm. Data are individual mice (spots) and mean (bars) of five mice separately assayed. Significant suppression of the D10.G4 clone was seen on co-culture with PEC that had been exposed to *B. malayi* E/S ( $P < 0.01$ ), or *T. canis* E/S ( $P < 0.05$ ).

This has led us to ask several questions regarding the *in vivo* relevance of this observation. Firstly, 'How does the parasite generate a suppressive host cell population?' The work described in this manuscript shows that host cells capable of blocking proliferation can be generated by the daily administration of adult *B. malayi* E/S. Notably, this suppression, like that of adult and L3 implants, cannot be reversed on the addition of nitric oxide inhibitors. These data, together with the inability of dead parasites to ablate proliferation, strongly point to the parasite producing a soluble factor(s) responsible for the subsequent development of a suppressive PEC population. Fractionation and chemical manipulation of the *Brugia* E/S will permit a practical approach to the identification of the parasite factor(s) involved in the recruitment or development of a down-regulatory host cell.

Secondly, 'How might a non-specific proliferative block lead to the antigen specific hyporesponsiveness seen in human infection?' Filarial parasites live in the afferent lymphatics where E/S products carried into the adjacent lymph node could directly effect lymphocyte function or maturation. We postulate that naive T cells migrating into the lymph node will first encounter filarial antigen in an environment in which they are unable to undergo normal cellular division. The impact of this is difficult to interpret directly but an active proliferative block may mimic what occurs in the absence of co-stimulation, allowing the accumulation of negative regulators in the cell and leading to an anergic state (Jenkins 1992). These cells, hypo-responsive to filarial antigen, would now enter the periphery.

One prediction of this model would be that at very high levels of infection, when adult parasites can be found at multiple sites, responses to non-parasite antigens would also be suppressed. In human studies of lymphatic filariasis, responses to non-filarial antigens are apparently intact (Yazdanbakhsh *et al.* 1993). However, PPD responses increase following chemotherapy suggesting there is an element of non-specific downregulation during infection (Sartono *et al.* 1995). In onchocerciasis, the evidence that responses to non-filarial antigens decrease with increasing infection is much stronger (Greene 1992 and J. Bradley, pers.comm. and M. Murdoch, unpublished). We are currently testing this model using naive T cells from T cell receptor transgenic mice to directly address the question of whether the proliferative block can lead to a state of anergy. Whether proliferative suppression plays a role in the generation of peripheral tolerance remains to be established, but it may directly facilitate L3 survival through the migratory phase and prevent extensive inflammatory responses to the adult stage within the lymphatic vessel.

Interestingly, other helminth studies have shown direct anti-proliferative effects of purified components of E/S or

concentrated E/S on lymphocytes *in vitro* (Harnett & Harnett 1993, Kadian *et al.* 1996, Cervi & Masih 1997). We have not observed any *in vitro* effect with either live *Brugia malayi* or unconcentrated E/S. The inability of *B. malayi* ES to prevent cellular proliferation *in vitro* may reflect the low concentration of the E/S used, although the potency of the same material *in vivo* argues that the concentrations used in our study are physiologically relevant. Alternatively, it could suggest that parasite-derived factors must act in concert with host immune system components to induce suppression. This is consistent with the requirement for IL-4 and our previous observation that one week of host exposure to the parasite is required for development of the proliferative block (MacDonald *et al.* 1998). Although filariae may directly suppress lymphocyte proliferation, the generation of a host cell population that produces an anti-proliferative factor may amplify the negative signal, thus resulting in a longer lasting, more profound inhibition of the host response.

Studies with cestode E/S have demonstrated a similar pattern of proliferative suppression (Rakha *et al.* 1991, Scitutto *et al.* 1995). This led us to question if the generation of a non-specific anti-proliferative host cell is a general phenomenon associated with helminth infection. We chose preliminary to look at two members within the Nematoda phylum distantly related to filarial parasites. We observed that E/S products from *Toxocara* and *Nippostrongylus* were able to reproduce the *in vivo* suppressive effect of *Brugia*. Unlike *Brugia*, the adult stage of both these nematodes lives primarily in the intestinal tract but like filarial nematodes, a larval stage must survive through a tissue migratory phase. These results suggest that the immuno-modulatory capacity of E/S may be a feature common to nematode infection presumably predicated on the ability of these organisms to induce host IL-4, as we have shown that the ability to generate a cell population capable of blocking proliferation does not occur in the absence of *in vivo* IL-4 (MacDonald *et al.* 1998). These studies will be extended to other helminths to further evaluate the universality of the observed phenomenon. It is possible that innate defence systems in the mammalian host can recognize features common to higher animals leading to a similarity in response to organisms that are phylogenetically quite distinct.

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#### REFERENCES

- Allen, J. E., Lawrence, R. A. & Maizels, R. M. (1995). Fine specificity of the genetically controlled immune response to native and recombinant gp15/400 (polyprotein allergen) of *Brugia malayi*. *Infection and Immunity* **63**, 2892–2898
- Allen, J. E., Lawrence, R. A. & Maizels, R. M. (1996). APC from mice harboring the filarial nematode, *Brugia malayi*, prevent cellular proliferation but not cytokine production. *International Immunology* **8**, 143–151
- Allen, J. E. & Maizels, R. M. (1997). Th1-Th2: reliable paradigm or dangerous dogma? *Immunology Today* **18**, 387–392
- Cervi, L. & Masih, D. T. (1997). Inhibition of spleen cell proliferative response to mitogens by excretory-secretory antigens of fasciola hepatica. *International Journal of Parasitology* **27**, 573–579
- Finkelman, F. D., Katona, I. M., Urban, J., *et al.* (1988). Interleukin-4 is required to generate and sustain *in vivo* IgE responses. *Journal of Immunology* **141**, 2335–2341
- Greene, B. M. (1992). Cellular and humoral immune response in onchocerciasis. *Journal of Infectious Disease* **165**, 1161
- Harnett, W. & Harnett, M. M. (1993). Inhibition of murine B-cell proliferation and down-regulation of protein kinase-C levels by a phosphorylcholine-containing filarial excretory-secretory product. *Journal of Immunology* **151**, 4829–4837
- Jenkins, M. K. (1992). The role of cell division in the induction of clonal anergy. *Immunology Today* **13**, 69–73
- Kadian, S. K., Dixon, J. B., Carter, S. D. & Jenkins, P. (1996). Macrophage modifying factor secreted by the tetrathyridia of *Mesocostoides corti* (cestoda): monoclonal antibody to the modifying factor antagonizes its immunological activity. *Parasite Immunology* **18**, 65–70
- Kaye, J., Porcelli, S., Tite, J. & Jones, B. (1983). Both a monoclonal antibody and antisera specific for determinants unique to individual cloned helper T cell lines can substitute for antigen and antigen-presenting cells in the activation of T cells. *Journal of Experimental Medicine* **158**, 836–856
- Lawrence, R. A. (1996). Lymphatic filariasis: what mice can tell us. *Parasitol Today* **12**, 267–271
- Lawrence, R. A., Allen, J. E., Gregory, W. F., Kopf, M. & Maizels, R. M. (1995). Infection of IL-4 deficient mice with the parasitic nematode *Brugia malayi* demonstrates that host resistance is not dependent on a Th2 dominated immune response. *Journal of Immunology* **154**, 5995–6001
- Lawrence, R. A., Allen, J. E., Osborne, J. & Maizels, R. M. (1994). Adult and microfilarial stages of the filarial parasite *Brugia malayi* stimulate contrasting cytokine and Ig isotype responses in BALB/c mice. *Journal of Immunology* **153**, 1216–1224
- Lawrence, R. A., Gray, C. A., Osborne, J. & Maizels, R. M. (1996). *Nippostrongylus brasiliensis*: cytokine responses and worm expulsion in normal and IL-4 deficient mice. *Experimental Parasitology* **84**, 65–73
- MacDonald, A. S., Maizels, R. M., Lawrence, R. A., Dransfield, I. & Allen, J. E. (1998). Requirement for *in vivo* production of IL-4, but not IL-10, in the production of proliferative suppression by filarial parasites. *Journal of Immunology* (in press)
- Maizels, R. M. & Lawrence, R. A. (1991). Immunological tolerance: the key feature in human filariasis? *Parasitology Today* **7**, 271–276
- Ottesen, E. A., Weller, P. F. & Heck, L. (1977). Specific cellular immune unresponsiveness in human filariasis. *Immunology* **33**, 413–421
- Piessens, W. F., McGreevy, P. B., Piessens, P. W., McGreevy, M.,

- Koiman, I., Saroso, J. S. & Dennis, D. T. (1980). Immune responses in human infections with *Brugia malayi*. Specific cellular unresponsiveness to filarial antigens. *Journal of Clinical Investigation* **65**, 172–179
- Rakha, N. K., Dixon, J. B., Skerritt, G. C., Carter, S. D., Jenkins, P. & Marshall-Clarke, S. (1991). Lymphoreticular responses to metacystodes: *Taenia multiceps* (Cestoda) can modify interaction between accessory cells and responder cells during lymphocyte activation. *Parasitology* **102**, 133–140
- Sartono, E., Kruize, Y. C. M., Kurniawan, A., van der Meide, P. H., Partono, F., Maizels, R. M. & Yazdanbakhsh, M. (1995). Elevated cellular immune responses and IFN- $\gamma$  release after long-term DEC treatment of patients with human lymphatic filariasis. *Journal of Infectious Diseases* **171**, 1683–1687
- Sciutto, E., Fragoso, G., Baca, M., de la Cruz, V., Lemus, L. & Lamoyi, E. (1995). Depressed T-cell proliferation associated with susceptibility to experimental *Taenia crassiceps* infection. *Infection and Immunology* **63**, 2277–2281
- Swain, S. L., Dennert, G., Warner, J. & Dutton, R. W. (1981). Culture supernatants of a stimulated T cell line have helper activity that acts synergistically with interleukin 2 in the response of B cells to antigen. *Proceedings of the National Academy of Sciences, USA* **78**, 2517–2521
- Yazdanbakhsh, M., Paxton, W. A., Kruize, Y. C. M., Sartono, E., Kurniawan, A., van het Wout, A., Selkirk, M. E., Partono, F. & Maizels, R. M. (1993). T cell responsiveness correlates differentially with antibody isotype levels in clinical and asymptomatic filariasis. *Journal of Infectious Diseases* **167**, 925–931