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Analysis of the mechanism of immunodepression following heterologous antigenic stimulation during concurrent infection with *Nematospiroides dubius*

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Summary. The suppression of immune responsiveness to heterologous antigenic stimulation during concurrent infection with *Nematospiroides dubius* was reproduced using soluble antigens derived from adult parasites. Immunosuppression appeared to be selective in that the administration of equivalent quantities of an irrelevant heterogeneous antigen had no immunosuppressive effect, and suppression was transferable using spleen cells from parasite antigen-treated donors. The differential immunomodulatory activity of parasite antigens from a variety of nematode species suggested that a correlation might exist between suppressor activity and chronicity of infection.

A role for suppressor T cell activity in the infected host was implicated by the restorative effect of 2′deoxyguanosine treatment on the immune response, and non-specific suppressor cell activity was detected in splenocyte populations from infected mice. It is suggested that a parasite-induced defect in antigen processing led to the induction of suppressor cell activity in the infected host and that this may be one mechanism of parasite survival. The relevance of these observations to vaccination against chronic gastrointestinal nematode infections is discussed.

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INTRODUCTION

Many species of protozoan and metazoan parasites have the ability to cause immunosuppression in their hosts (Floersheim, 1982; Playfair, 1982) and it has been suggested that immunosuppression is one mechanism by which some parasites facilitate their own survival (Ogilvie & Wilson, 1976). It is, therefore, important that immunoparasitologists utilize models of chronic parasite infection and immunosuppression to the full in order that a better understanding of potential immunogenic and immunomodulatory parasite products can be achieved. The trichostrongyle nematode *Nematospiroides dubius* survives in the mouse duodenum for 8 months or more in a primary infection (Ehrenford, 1954), and immune responses to a variety of heterologous antigens such as sheep erythrocytes (Shimp, Crandall and Crandall, 1975; Ali & Behnke, 1983), viruses (Chowaniec, Wescott & Congdon, 1972) and other nematode parasites (Behnke, Wakelin & Wilson, 1978) are suppressed during infection. The demonstration that adult *N. dubius* can interfere with the host's ability to express the homologous immunity generated by irradiated infective larvae (Behnke, Hannah and Pritchard, 1983) is of particular relevance to the elucidation of the mechanism of parasite survival.

However, no firm experimental evidence of immunosuppression by soluble antigens of *N. dubius*

exists, although the latter study would suggest that adult *N. dubius* are capable of producing immunomodulatory factors (IMF). The present study was therefore initiated to study the immunomodulatory potential of adult *N. dubius* antigen, and to investigate mechanisms by which possible IMF function in the parasitized host. The timing of antigen injection was designed to mimic a duration of adult worm residence in the intestine which is known to be immunodepressive against immune responsiveness to sheep erythrocytes (Ali & Behnke, 1983), whilst the potential of IMF to generate suppressor cell activity was assayed using the documented and reliable system of Whisler & Stobo (1976). Therefore, whilst the primary objective of the study was to assess the potential of parasite-derived antigens to modulate immunity, the work was also extended to investigate the presence of suppressor cells in the infected hosts and mechanisms by which suppressor cell activity could have been generated. The strain difference of immune responsiveness to *N. dubius* (Behnke & Wakelin, 1977) also warranted an investigation into the ability of NIH and C57 B/10 mice to generate suppressor cell activity.

MATERIALS AND METHODS

Animals and experimental infection

Inbred NIH and C57 B/10 and randomly bred CFLP mice were bred and maintained under conventional conditions in the departmental animal house. Infective third-stage larvae of *N. dubius* were given by stomach intubation in a volume of 0·2 ml. The methods used for the maintenance and recovery of *N. dubius* have been described previously (Jenkins & Behnke, 1977).

Antigen preparation

Adult *N. dubius* antigen was prepared from adult worm homogenate (AH). Following homogenization in sterile 0·9% saline the suspension was centrifuged at 10,000 rev/min at 4° for 1 hr and the supernatant recovered. The protein concentration was determined using the Bio-Rad Assay system (Bio-Rad, U.K.) and adjusted to a stock concentration of 10 mg/ml.

Plaque-forming cells (PFC)

The methods used for the determination of direct plaque-forming cells have been described previously (Ali & Behnke, 1983). Briefly, PFC assay chambers were prepared on microscope slides (Cunningham & Szenberg, 1968). Spleens were removed from individual animals and teased apart on fine mesh stainless-steel grids. Cell suspensions were washed in Hanks's balanced salt solution containing five units of heparin/ml and kept at 4° until required. The indicator sheep erythrocyte cell suspension was prepared as described by Marbrook, Mishell & Shiigi (1980).

Suppressor cell activity

The methods used for the assessment of suppressor cell activity were essentially as described by Whisler & Stobo (1976). Homologous suppressor cells to sheep erythrocytes were generated by the hyperimmunization of mice with 2×10^8 to 2×10^{10} sheep erythrocytes intraperitoneally. Fourteen days later, spleens were removed, single cell suspensions made and 5×10^7 'suppressor cells' injected intravenously (i.v.) into syngeneic recipients. These recipients were challenged with 5×10^7 sheep erythrocytes and the number of direct PFCs determined 4 days later.

To test the ability of adult *N. dubius* antigen to generate suppressor cell activity to sheep erythrocytes, donor mice were injected i.v. with adult homogenate for 6 days. On the 3rd day of the injection schedule, the donors were challenged with 5×10^7 sheep erythrocytes and direct PFCs assayed 4 days later. Spleen cells removed from AH-injected animals 4 or 14 days following challenge with sheep erythrocytes were assayed for suppressor cell activity on transfer into syngeneic recipients, in the manner described above.

Spleen cells, thymocytes and mesenteric lymph node lymphocytes were assessed for non-specific (heterologous) suppressor cell activity 14 days following infection of NIH (400 L3) mice and C57 B/10 (250 L3) mice. Single cell suspensions (10^8) were injected intraperitoneally on the same day as challenge with 5×10^7 sheep erythrocytes. The direct plaque-forming cell response was assessed 4 days later.

Administration of 2′deoxyguanosine

Deoxyguanosine (Sigma), an inhibitor of T suppressor cell activity (Dosch *et al.*, 1980), was dissolved in sterile saline and administered intraperitoneally (i.p.) at a dose rate of 1 mg/day for the duration of infection.

Macrophage studies

Macrophage function in mice infected with *N. dubius* was assessed using the system described by Loose,

Cook & Di Luzio (1971). Normal and infected (250 L3) C57 B/10 mice were treated with 2 ml of 5% thioglycolate broth (i.p.) 14 days following infection. Four days later mice were injected with 5×10^7 sheep erythrocytes i.p. and peritoneal lavage leucocytes harvested 3 hours later using 5 ml Hanks's saline with 5 IU/ml heparin. Free sheep erythrocytes were lysed with distilled water and erythrocyte-containing peritoneal leucocytes (1×10^7) injected either i.p. or i.v. into syngeneic recipients. Plaque-forming cells were assayed 4 days following injection.

Macrophage phagocytosis was assayed visually following sheep erythrocyte immunization into the peritoneum using cytocentrifuge preparations stained with Leishman-Giemsa, and quantitatively using FITC-coated latex beads. Macrophages from normal and infected donors (d14) were obtained from non-induced peritoneal exudates by peritoneal lavage with 5 ml TC medium 199 containing *L*-glutamine, 5% foetal calf serum (FCS) and buffered with Hepes. The medium also contained 5 IU heparin/ml. Individual peritoneal lavages were adjusted to give a final concentration of 1×10^6 leucocytes/ml and one million cells from each sample pelleted by centrifugation. Ten μl of a 1/10 dilution of FITC latex stock (Gibco) was added to each pellet and the volume made up to 1 ml with mixing. Following incubation at 37° for 45 min, samples were washed three times in medium to remove free beads and each pellet finally reconstituted to 1 ml. The percentage of phagocytosing cells was determined using a Becton Dickinson FACS system and the degree of phagocytosis determined visually using cytocentrifuge preparations.

Expression and statistical analysis of results

The direct plaque-forming cell (PFC) response is expressed as the number of PFC/spleen $\pm$ 1 SE. Results were analysed for significance using Student's *t*-test. A probability of $P < 0{\cdot}05$ was considered significant.

RESULTS

1. The generation of homologous suppressor cells in C57 B/10 and NIH mice

Initial experiments were conducted to determine the suitability of both C57 B/10 and NIH mice for studies on suppressor cell activity. Female mice were pre-immunized with 2×10^8, 2×10^9 or 2×10^{10} sheep erythrocytes i.p. Fourteen days following immunization their spleens were removed and cell suspensions prepared; 5×10^7 spleen cells were injected i.v. into syngeneic recipients. An additional recipient group received 5×10^7 normal non-immune spleen cells. Each recipient group received 5×10^7 sheep erythrocytes i.p. on the same day, and the direct PFC response was assessed 4 days later (Fig. 1).

It can be seen from Fig. 1 that C57 B/10 mice generated suppressor cell activity more readily than NIH mice, and were generally poor responders to

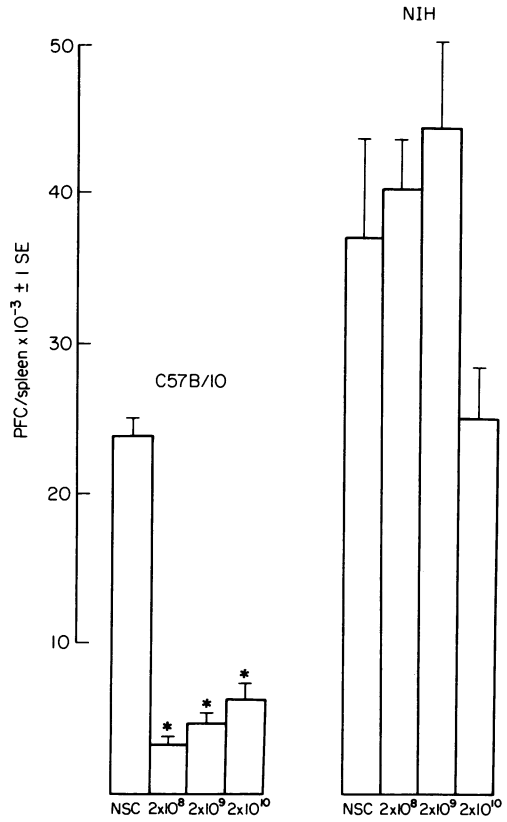

Figure 1. The generation of homologous suppressor cells to sheep erythrocytes in C57 B/10 and NIH mice. Female NIH and C57 B/10 mice ($n = 5$) were immunized i.p. with 2×10^8, 2×10^9 or 2×10^{10} sheep erythrocytes. Fourteen days later, spleens were removed and single cell suspensions made. 5×10^7 viable spleen cells from each group were injected i.v. via the lateral tail vein into syngeneic groups of recipients. An additional recipient group received normal spleen cells (NSC). Each mouse was injected i.p. with 5×10^7 sheep erythrocytes on the day of transfer and the direct PFC response assessed 4 days later. (Statistical analysis: $*P < 0{\cdot}001$ *vs* control (NSC).)

sheep erythrocytes compared to NIH mice. C57 B/10 mice produced on average 23,680 direct PFC/spleen 4 days following immunization, whilst NIH mice produced 36,960 direct PFC/spleen. The response of C57 B/10 mice to sheep erythrocytes was significantly suppressed following the transfer of 5×10^7 spleen cells from syngeneic donors pre-immunized with as few as 2×10^8 sheep erythrocytes, whilst NIH mice required 5×10^7 spleen cells from donors pre-immunized with 2×10^{10} sheep erythrocytes before suppression was seen (Fig. 1). It is important to note here that this differential degree of suppression between the strains was also evident in the suppressor cell donors following immunization with 2×10^{10} sheep erythrocytes, indicating that it was unlikely that immunity was being transferred (with a subsequent depression in the IgM PFC response). This experiment has been repeated on three separate occasions with similar results and therefore all subsequent experiments were carried out on C57 B/10 mice.

2. The effect of adult-derived antigens of *N. dubius* on the direct plaque-forming cell response to sheep erythrocytes, and the ability of these antigens to generate or facilitate suppressor cell activity

To assess the effect of adult *N. dubius* antigen on the direct PFC response to sheep erythrocytes, female C57 B/10 mice were injected i.v. daily for 6 days with 2 mg of adult *N. dubius* homogenate or 2 mg heat-inactivated FCS as control. The daily dosage was determined following a dose-response experiment, the

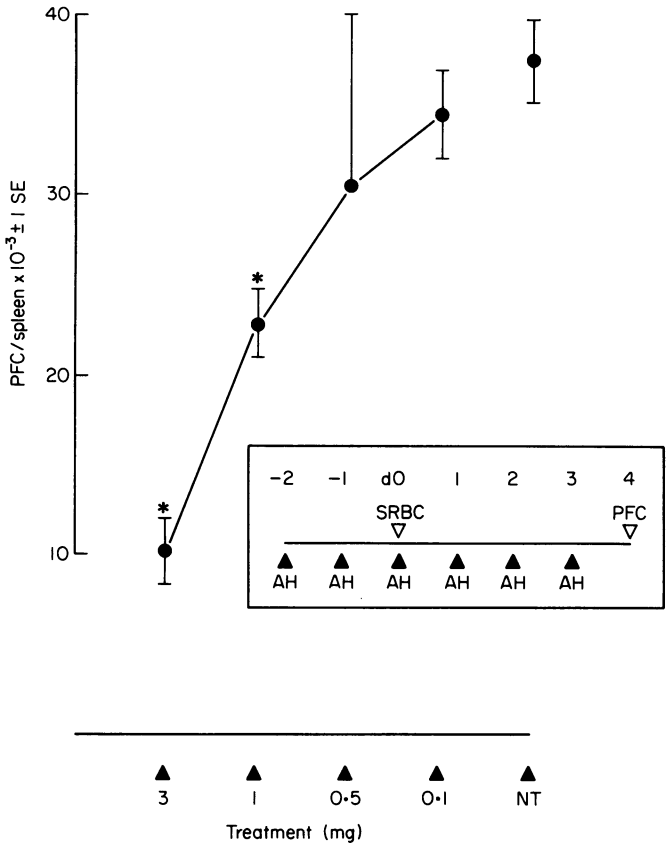

Figure 2. The dose-response of the suppressive effect of infusion with adult *N. dubius* homogenate (AH) on the direct PFC response to sheep erythrocytes. Female C57 B/10 mice ($n = 6$) were injected i.v. for 6 days with adult *N. dubius* homogenate (AH). The experimental plan is shown as an inset. The direct PFC response of each group was assessed 4 days following the injection of 5×10^7 sheep erythrocytes and compared with that of a non-treated but immunized control group (NT). (Statistical analysis: $^*P < 0{\cdot}005$ *vs* control (NT).)

results of which are shown in Fig. 2, and the schedule of injections was designed to mimic infection. Mice infected for 14 days with *N. dubius* have reduced responsiveness to immunization with sheep erythrocytes. As adults are present from 10 days following infection, the pattern of injection closely resembles the term of adult worm presence in the intestine relative to the timing of immunization. The total direct plaque-forming cell response was assessed 4 days following immunization with 5×10^7 sheep erythrocytes.

Spleen cells were also transferred into syngeneic recipients 4 or 14 days following immunization with sheep erythrocytes to investigate the ability of AH to generate or facilitate suppressor cell activity. A summary of the complete experimental plan is shown in Fig. 3.

Treatment with adult homogenate significantly suppressed the immune response to sheep erythrocytes, whilst an alternative and equally heterogeneous antigen, FCS, given in identical quantities, had no suppressive effect (Fig. 4). Treatment with adult homogenate reduced the number of plaque-forming cells by 80% from 19,933 to 4000, whilst the mean number of plaques from animals treated with FCS was 23,833. This effect is highly reproducible and has been repeated on a number of separate occasions. The degree of reduction induced by adult homogenate was comparable to that observed in animals infected with 300 L3 larvae of *N. dubius* 14 days prior to immunization with sheep erythrocytes (73·4%—Ali & Behnke, 1983). It is also worthy of note that animals treated with adult homogenate or infection had pronounced splenomegaly.

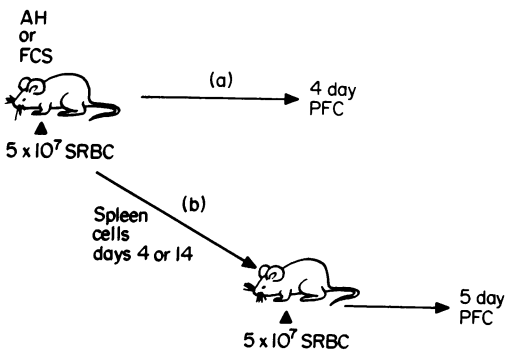

Figure 3. A summary plan of the experiments shown in Figs 2 and 4. (a) Mice treated with adult *N. dubius* homogenate (AH) or fetal calf serum (FCS) were assessed for their ability to produce direct plaque-forming cells 4 days following immunization with 5×10^7 sheep erythrocytes. (b) 5×10^7 spleen cells from animals treated with adult homogenate were transferred 4 or 14 days following immunization with sheep erythrocytes, and the effect of this transfer on the response of recipients to sheep erythrocytes assessed.

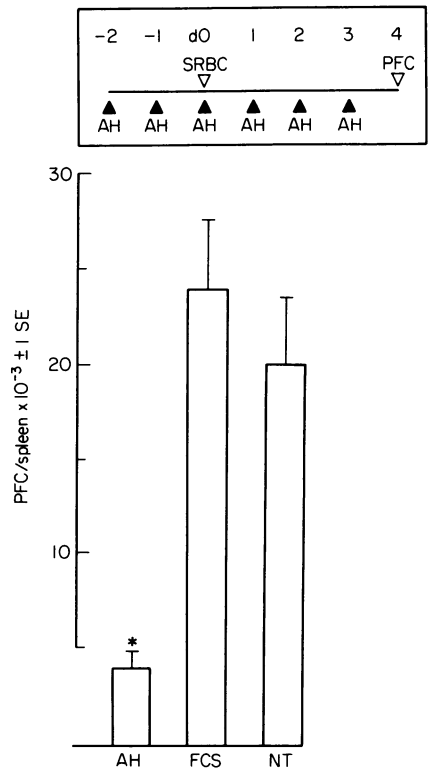

Figure 4. The suppressive effect of adult *N. dubius* homogenate (AH) compared with that of an irrelevant antigen administered in equal quantities. Female C57 B/10 mice ($n = 6$) were injected i.v. for 6 days with 2 mg of adult *N. dubius* homogenate (AH) or 2 mg of fetal calf serum (FCS). The experimental plan is shown as an inset. The direct plaque-forming cell response of each group was assessed 4 days following the injection of 5×10^7 sheep erythrocytes (SRBC) and compared with that of a non-treated but immunized control group (NT). (Statistical analysis: $^*P < 0{\cdot}005$ *vs* control (NT).)

In a subsequent experiment to assess the possible contribution of antigenic competition, it has been shown that treatment with *N. dubius* homogenate (1 mg/day) caused a 56% reduction in the immune response to sheep erythrocytes ($P < 0{\cdot}005$), whilst similar quantities of *Ascaris suum* adult antigen caused a 46·4% reduction ($P < 0{\cdot}005$), *Trichinella spiralis* L_1 antigen a 16·7% reduction ($P < 0{\cdot}05$) and *N. brasiliensis* adult homogenate a 132% enhancement ($P < 0{\cdot}01$).

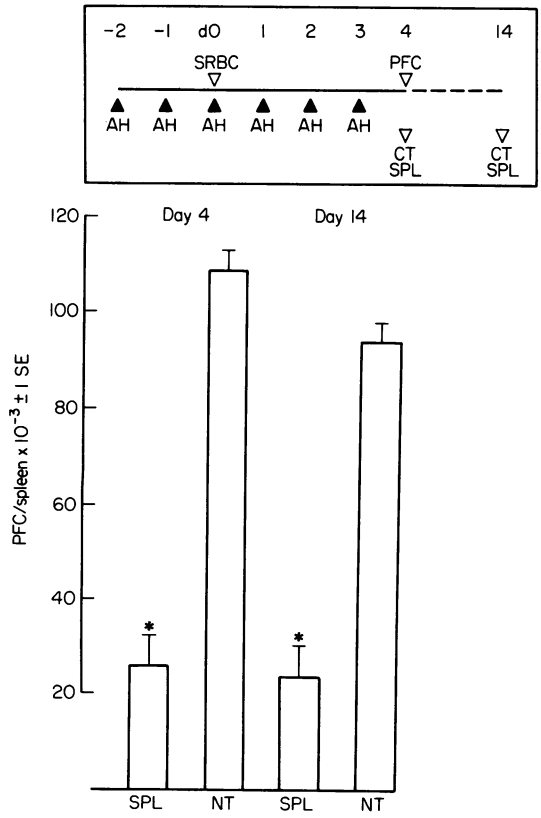

Figure 5. The generation of suppressor cell activity following the injection of adult homogenate of *N. dubius*. 5×10^7 spleen cells were transferred from AH-treated donors either 4 or 14 days following the injection of sheep erythrocytes (see inset and Fig. 3). One day later, recipients of spleen cells (SPL) were injected i.p. ($n = 8$) with 5×10^7 sheep erythrocytes. The direct plaque-forming cell response of each group was assessed 5 days following the injection of sheep erythrocytes and compared with that of a non-treated but immunized control group (NT). (Statistical analysis: $*P < 0{\cdot}005$ *vs* control (NT).)

Suppression of the immune response to sheep erythrocytes by adult homogenate was transferrable to naive recipients using spleen cells from AH-treated donors (Fig. 5). For convenience, the total number of plaque-forming cells were assessed 5 days following immunization with sheep erythrocytes in this instance, and as a result a greater number of total PFC/spleen were observed. However, the injection of 5×10^7 spleen cells from AH-treated donors prior to immunization with sheep erythrocytes resulted in a dramatic reduction in the number of total PFC recorded. Spleen cells transferred from AH-treated donors 4 days following immunization with sheep erythrocytes caused a 76% reduction, whilst cells transferred 14 days following immunization caused a 75% reduction in total PFC recorded.

3. The effect of treatment with deoxyguanosine on the suppression of the immune response to SRBC following infection with *N. dubius*

Infection with 200 larvae of *N. dubius* depressed the immune response to SRBC by 73% with the number of direct PFC/spleen being reduced from 31,900 in the control group to 7520 in the infected group. Although deoxyguanosine treatment had little effect on the immune response to SRBC (27,640 direct

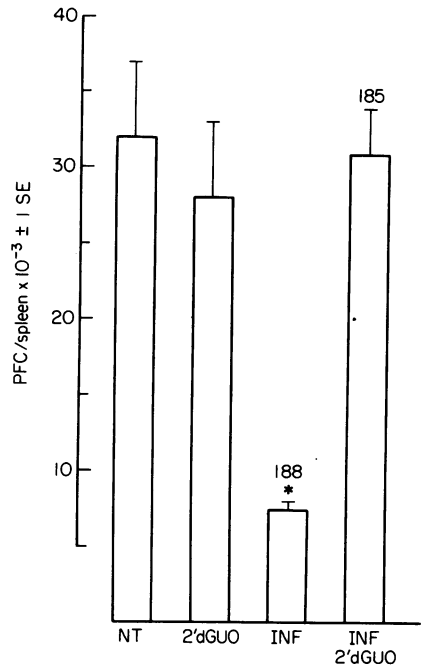

Figure 6. The effect of 2'deoxyguanosine (2'dGUO) on immunosuppression generated by infection with 200 L3 larvae of *N. dubius*. Female C57 B/10 mice were infected on day 0 of the experiment, immunized i.p. with 5×10^7 sheep erythrocytes on day 14, and their direct PFC response assessed on day 18 (INF, $n = 10$). A second group (INF, 2'dGUO, $n = 10$) was infected and immunized as above but treated daily (1 mg/day i.p.) with deoxyguanosine. The number of PFC/spleen generated was compared with an immunized non-infected group (NT, $n = 5$) and an immunized non-infected group treated with deoxyguanosine (d'dGUO, $n = 5$). Worm recoveries from infected groups are shown above the error bars. (Statistical analysis: $*P < 0{\cdot}005$ *vs* all other groups.)

PFC/spleen), a combination of deoxyguanosine treatment and infection restored the number of PFC/spleen to control levels (30,820 PFC/spleen). Deoxyguanosine treatment had no significant effect on worm recovery, with a mean of 188 worms recovered from the infected group, and a mean of 185 recovered from the infected group treated with deoxyguanosine (Fig. 6).

4. Non-specific (heterologous) suppressor cells in mice infected with *N. dubius*

The transfer of splenocytes from mice undergoing a chronic primary infection with *N. dubius* had a significant effect on the immune response to sheep erythrocytes in recipient groups (Table 1). However, mesenteric lymph node cells and thymocytes failed to suppress the immune response when given in equivalent numbers.

Table 1. Non-specific (heterologous) suppressor cells in mice infected with *N. dubius*

	C57 B/10 PFC/spleen ± 1 SE
NT	75,200 ± 1960
Thymocytes	79,800 ± 3300
Mesenteric lymph node (MLN) cells	73,320 ± 3840
*10^8 splenocytes	32,000 ± 1608
*2×10^8 splenocytes	22,600 ± 816

* Statistically significant ($P < 0{\cdot}001$).

Lymphoid cells were transferred into syngeneic recipients 14 days following infection of female donor C57 B/10 (200 L3) mice. The effect of transfer on the direct PFC response to 5×10^7 sheep erythrocytes given on the same day as transfer was assessed 4 days later ($n = 6$).

5. Macrophage function in mice infected with *N. dubius*

To assess macrophage function, female C57 B/10 mice were injected i.p. with 5×10^7 sheep erythrocytes and peritoneal leucocytes were recovered by lavage 3 hr later. A leucocyte preparation (1×10^7) including erythrocyte-containing macrophages was injected i.v. or i.p. into syngeneic recipients and the direct PFC response was measured 4 days later.

It can be seen from Table 2 that macrophages recovered from the peritoneal cavities of mice infected for 14 days with *N. dubius* had a diminished capacity to transfer immunity to sheep erythrocytes compared with macrophages from normal animals, despite the

Table 2. Depression of the PFC response to SRBC in recipient mice injected with peritoneal leucocytes from SRBC-primed syngeneic donors infected with *N. dubius*

Control (5×10^7) SRBC i.p.)	1×10^7 normal macrophages i.p.	1×10^7 normal macrophages i.v.	1×10^7 'infected' macrophages i.p.	1×10^7 'infected' macrophages i.v.
24,000	650	2350	475	500
30,400	950	2050	275	500
18,000	1350	2800	375	250
23,200	1600	2800	200	350
—	1100	2450	625	400
*23,900 ± 2542	1130 ± 163 a	2490 ± 143 c	390 ± 75 b	400 ± 47 d

* No. of PFC/spleen ± 1 SE.

Statistical analysis: a *vs* b, c *vs* d, $P < 0{\cdot}001$.

C57 B/10 mice were injected i.p. with 5×10^7 sheep erythrocytes. Three hours later, peritoneal leucocytes were harvested and 1×10^7 leucocytes from normal or infected donors injected either i.p. or i.v. into syngeneic recipients. The number of PFC/spleen was determined 4 days later.

Table 3. FACS analysis of FITC-latex uptake by macrophages harvested from peritoneal cavities of normal or infected (250 L3) C57 B/10 mice

		% phagocytic cells (FACS —mean ± 1 S.E.)		Differential leucocyte composition LY.	M	MAST.	EOS.	POLY.
Normal	1	27·8		24	75	1	0	0
	2	28·4		9	87	3	1	0
	3	46·2	37·3 ± 4·0	10	89	1	0	0
	4	38·3		30	69	1	0	0
	5	45·6		25	72	1	1	0
Infected	1	49·1		11	86	0	2	1
	2	44·6		22	78	0	0	0
	3	40·9	42·8 ± 4·4	23	77	0	0	0
	4	27·1		12	87	0	1	0
	5	52·6		5	95	0	0	0

Non-induced macrophages were harvested from mice 14 days following infection with 250 L3 larvae of *N. dubius* and their ability to phagocytose FITC-coated latex beads compared with macrophages from non-infected mice using a fluorescence-activated cell sorter (FACS). The differential leucocyte composition of each preparation is included for comparison.

fact that visual assessment revealed that macrophages from infected animals contained an equivalent number of sheep erythrocytes. The latter observation was quantitated and confirmed by FACS analysis following the *in vitro* phagocytosis of fluorescent latex beads by unstimulated macrophages from both normal and infected donors. A comparable phagocytic index was obtained for both normal and infected macrophages following the uptake of fluorescent latex beads (Table 3). It is also worthy of note that the differential leucocyte composition of samples from each group was comparable.

DISCUSSION

The phenomenon of chronic gastrointestinal nematode infection provides an intriguing challenge to the immunoparasitologist because the mechanisms which parasites use to evade immunity are both intricate and diverse in nature (Ogilvie & Wilson, 1976). The mouse trichostrongyle, *N. dubius*, survives in the duodenum for 8 or more months during a primary infection (Ehrenford, 1954) and ultimate rejection is almost certainly due to the senescence of the parasite (Williams & Behnke, 1983). The parasite's ability to survive during the preceding months is therefore of great interest, particularly as it is known that an immune response can be generated against *N. dubius* under certain circumstances (Behnke & Parish, 1979, 1981). However, despite the intensity of research on immunity to *N. dubius*, little is known of the mechanisms of survival of this nematode, and information obtained using this model could lead to valuable application in the field of human immunoparasitology.

In the present study it has been demonstrated unequivocally that adult-derived antigens of *N. dubius* are capable of suppressing an immune response to a heterologous antigen, in this case sheep erythrocytes. This observation is in agreement with the work of Shimp *et al.* (1975) and Ali & Behnke (1983), who demonstrated the immunosuppressive effect of infection with *N. dubius* on haemagglutination titres and the direct PFC response following concurrent immunization with sheep erythrocytes. The ability of adult antigens to modulate an immune response also supports the hypothesis that adult *N. dubius* are capable of producing immunomodulatory factors (IMF) *in vivo* which suppress the immune system. It is now relevant to determine whether adult antigens are capable of suppressing the immunity generated by irradiated *N. dubius* larvae as effectively as adults transferred to the immunized host by laparotomy (Behnke, Hannah & Pritchard, 1983). In addition, the pronounced suppressive effect of adult homogenate should allow the exact nature of the immunosuppressive factor to be determined.

The failure of other workers to immunize against *N. dubius* using crude antigen preparations may be explained by the presence of immunosuppressive substances in their immunogens. However, Day *et al.* (1979) did demonstrate the immunogenic potential of adults by implanting them into the peritoneal cavity, and adult antigens are recognized by protective IgG_1 immunoglobulin isolated from immune serum by immunochemical means (Pritchard *et al.*, 1983). It would, therefore, appear that adult *N. dubius* is capable of both immunization or suppression and that the latter situation prevails during a chronic primary infection. The molecular heterogeneity of adult *N. dubius* homogenate is well documented (Day *et al.*, 1979; Pritchard *et al.*, 1983) although it remains to be seen whether the immunogenic and suppressive capabilities are distinct. However, the importance of this approach to the production of immunogenic vaccines cannot be underestimated.

If *N. dubius* survives as a chronic infection by the production of an immunosuppressive substance then the mechanism of suppression must be subtle enough to prevent the demise of the host through opportunist infections. However, as stated earlier, the mechanisms by which parasites evade immunity are both intricate and diverse, and so each is discussed in relation to infection with *N. dubius*.

The mechanism by which *N. dubius* homogenate causes immunosuppression, in this case to a heterologous antigen, is unlikely to be related to antigenic competition as the injection of a comparably heterogenous antigen (FCS) had no significant effect on the number of direct plaque-forming cells. It was also noted that antigens from a variety of species of parasitic helminth had differential effects on the immune response, indicating that parasite products may have true intrinsic immunomodulatory activity. The enhancement of immunity by *N. brasiliensis* was particularly interesting in this respect, although this observation is not entirely new (Vickery, Klein & Friedman, 1980).

The ability of spleen cells from animals injected with adult homogenate to transfer suppression to naive recipients indicates that the suppression induced by

adult homogenate was actively cell-mediated and agrees with the observation that splenocytes from infected mice have suppressor activity. The pronounced restorative effect of deoxyguanosine treatment on the immune response to sheep erythrocytes during concurrent infection with *N. dubius* provides further evidence of a role for suppressor cells in this system. Deoxyguanosine administration has been shown to inhibit suppressor T cell development (Dosch *et al.*, 1980) and experiments are in progress to determine the exact phenotype of the suppressor cell induced during infection with *N. dubius*.

Suppressor cell activity in parasite infections is widespread (reviewed by Playfair, 1982), and it would appear that parasite-induced suppressor cells can act in both an antigen specific and non-antigen specific manner depending on the system studied. For example, antigen-specific suppressor T cells are active during chronic *T. cruzi* infection in mice (Scott, 1981), whilst Lelchuck, Sprott & Playfair (1981) in contrast have demonstrated the presence of non-specific suppressor T cells in *P. berghei* infected mice. An interesting parallel can be drawn between *P. berghei*-induced suppression and *N. dubius*-induced suppression in that heterologous suppression against an immune response to sheep erythrocytes can in each case be demonstrated using soluble products of infection; in *P. berghei*-infected mice a suppressive component of high molecular weight (150,000) was isolated from the blood of heavily infected mice (Liew, Dhaliwal & Teh, 1979).

It is possible that the *N. dubius*-infected mouse provides an environment for the generation of suppressor cells to any incoming heterologous antigenic stimulation. Normal antigen processing by macrophages has been shown to be affected by chronic parasite infection (Loose *et al.*, 1971) and appeared to be defective following infection with *N. dubius* (although phagocytosis was unaffected), making it conceivable that parasite-induced defects in antigen presentation led to inappropriate presentation of heterologous antigen and the generation of suppressor cells in *N. dubius*-infected mice (Spitznagel & Alison, 1970; Katz & Unanue, 1973). However, the failure of deoxyguanosine treatment to affect parasite survival in the face of restored immunity to sheep erythrocytes suggests that suppressor cells may not be the sole reason for parasite survival.

To conclude, it has been demonstrated that adult antigens of *N. dubius* suppress the immune system and that suppression is cell-mediated. Suppressor cell activity also occurs in the infected host and possibly acts to promote parasite survival. The generation of suppressor cell activity is associated with defective antigen processing and *in-vitro* studies are planned to clarify this point. Finally, the ability of antigens released during a chronic intestinal nematode infection to immunosuppress indicates that great care will have to be taken in the preparation of vaccines to other neamtode infections of veterinary and medical importance.

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