Clin. exp. Immunol. (1984) **56,** 337–344.

Immune responsiveness following intratracheal inoculation with *Histoplasma capsulatum* yeast cells

DEBORAH A. NICKERSON & P. FAIRCLOUGH *Department of Biology, University of South Florida, Tampa, Florida, USA*

(Accepted for publication 22 December 1983)

SUMMARY

Splenic *in vitro* immune responses from C57Bl/6 mice have been evaluated after intratracheal (i.t.) or intravenous (i.v.) inoculation with live *Histoplasma capsulatum* yeast cells. Significant ($P < 0{\cdot}005$) numbers of cells reactive to heat killed *H. capsulatum* (HK-Hc) were present 1 week after i.t. inoculation with 5×10^5 viable yeast cells. In contrast, blastogenic responses to HK-Hc were not detected until 3 weeks after i.v. *H. capsulatum* inoculation. Examination of non-specific immunity, i.e. mixed lymphocyte responses to alloantigens and antibody formation to sheep erythrocytes, revealed a significant ($P < 0{\cdot}005$) and transient depression of these responses 1 week following i.t. *H. capsulatum* inoculation. The non-specific decreases associated with early (1 week) i.t. infection were not mediated by suppressor cells but were related to the number of viable yeast administered.

Keywords intratrachael histoplasmosis immunity yeast cells

INTRODUCTION

Histoplasma capsulatum is a biphasic fungus capable of producing serious human infection, i.e. chronic pulmonary and disseminated histoplasmosis (Goodwin & Des Pres, 1978; Reddy *et al.*, 1976; Smith & Utz, 1972). Measurement of specific and non-specific immunity in patients during histoplasmoisis has revealed a wide spectrum of *in vivo* and *in vitro* reactivity. These studies have indicated that some patients with histoplasmosis can exhibit immune responses within the normal range, while others demonstrate significantly lowered immune potential to several different antigens (Alford & Goodwin, 1972; Cox, 1979; Newberry *et al.*, 1968; Stobo *et al.*, 1976). In certain instances, the immune depression observed during histoplasmosis has been correlated with the presence of suppressive cells (Stobo *et al.*, 1976) or serum factors (Cox, 1979; Newberry *et al.*, 1968).

Recent experimental studies have demonstrated that non-specific immune depression accompanies the early disease process in murine self-limiting histoplasmosis induced by either intravenous (i.v.) (Artz & Bullock, 1979b; Nickerson, Havens & Bullock, 1981) or subcutaneous (s.c.) (Tewari *et al.*, 1982) inoculation of *H. capsulatum* yeast cells. Moreover, i.v. yeast cell inoculation in C3H/ANF or C57Bl/6 mice consistently led to the development of potent non-specific suppressor cells (Nickerson *et al.*, 1981; Watson & Bullock, 1982). The fact that immune depression or suppression is not a consistent clinical observation indicates that other models of experimentally-induced histoplasmosis should be explored. In the present study, we have evaluated the *in vitro* immune potential of C57Bl/6 mice after intratracheal (i.t.) or i.v. inoculation of *H. capsulatum* yeast cells.

Correspondence: Dr Deborah A. Nickerson, Department of Biology, University of South Florida, Tampa, Florida 33620, USA.

MATERIALS AND METHODS

Animals. Male C57Bl/6 mice, 5–6 weeks of age were obtained from Jackson Laboratories (Bar Harbor, Maine, USA). Male BALB/c mice were bred in our animal facilities and used at 8–12 weeks of age. All mice were maintained under standard conditions and given food and water *ad libitum.*

Preparation of H. capsulatum *and animal inoculation.* Yeast cells of *H. capsulatum* strain G217-B were maintained at 37°C by weekly transfer on brain heart infusion (BHI) agar supplemented with 5% sheep red blood cells (SRBC). Standardized preparations of *H. capsulatum* were obtained as previously described by Artz & Bullock (1979a). Briefly, yeast cells were grown in BHI broth supplemented with 100 units/ml penicillin and 100 μg/ml streptomycin at 37°C on a rotary shaker (175 r/min). Cells were harvested after 36 h, washed three times with Hank's balanced salt solution (HBSS), and large aggregates removed by a final centrifugation at 30*g* for 1 min. Yeast cells were counted in a haemacytometer and adjusted to the appropriate cell concentrations. Mice were inoculated with 0·05 ml of the yeast cell suspension by either the i.t. or i.v. route. For i.t. inoculations, mice were anaesthetized with sodium pentobarbital and the tracheae exposed for immunization. Controls received 0·05 ml of HBSS.

Preparation of spleen cells. Spleens were removed aseptically and placed into Petri dishes containing HBSS. Single cell suspensions were prepared by gently teasing the spleen between two ground glass slides in HBSS. The large debris was removed by sedimentation at 1*g* for 5 min. Cell suspensions were obtained from pools of three to five animals. Spleen cells were washed three times in HBSS and resuspended into the appropriate culture medium.

Lymphocyte transformation assays. Spleen cells (5×10^5) were cultured in RPMI 1640 supplemented with 2% fetal calf serum (FCS), 100 units/ml penicillin, 100 μg/ml streptomycin, 1 mM glutamine and 50 units/ml mycostatin (GIBCO, Grand Island, New York, USA) in one well of a flat bottom microtitre plate (96 wells, Costar, Cambridge, Massachusetts, USA). Antigen, 5×10^5 heat killed *H. capsulatum* (HK-Hc; 60°C, 60 min) yeast cells in 0·1 m of supplemented RPMI 1640 was added to triplicate wells. Control wells received 0·1 ml of the supplemented medium. Cultures were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2 for 96 h.

Eighteen hours prior to harvest, 1 μCi of ^{3}H-thymidine (ICN Pharmaceuticals, Irvine, California, USA) in 0·02 ml of RPMI 1640 was added to each well. Cells were collected onto glass fibre filter paper using an automatic cell harvestor (MASH; Microbiological Associates, Walkersville, Maryland, USA) and the levels of ^{3}H-thymidine incorporation determined in a liquid scintillation counter. Results are expressed as the mean counts per minute (ct/min) for experimental (antigen) minus control (media alone) for triplicate cultures. Counts for control (media alone) cultures (normal or *H. capsulatum* inoculated animals) ranged from 2·8 to $7{\cdot}8 \times 10^3$ ct/min.

Mixed lymphocyte assay. In vitro lymphocyte reactions (MLR) were measured by a slight modification of the procedure described by Simpson *et al.* (1975). Stimulator spleen cells from BALB/c (H-2^d) were treated with 50 μg/ml mitomycin C for 35 min at 37°C. Stimulator cells were then washed four times in HBSS, adjusted to 5×10^6 cells/ml in RPMI 1640 supplemented with 10% FCS, 5×10^{-5} M 2-mercaptoethanol, 100 units/ml penicillin, 100 μg/ml streptomycin, 1 mM glutamine, 50 units/ml mycostatin and 0·1 ml added to a well of a flat bottom microtitre plate (96 wells). Responder (C57Bl/6) splenocytes (5×10^6/ml) were added to stimulator cells in 0·1 ml volumes. Control cultures consisted of responder splenocytes (5×10^5) mixed with mitomycin C treated responder splenocytes (5×10^5). Cultures were incubated at 37°C in a humidified 5% CO_2 air atmosphere for 96 h. Eighteen hours prior to harvest, cultures were pulsed with 1 μCi ^{3}H-thymidine. Cultures were collected onto filters and radioactivity counted in a liquid scintillation spectrophotometer. Results are expressed as the mean number of ct/min for experimental (responder + mitomycin C treated stimulator cells) cultures minus control (responder + mitomycin C treated responder cells) cultures. Counts for control cultures ranged from 3·4 to $9{\cdot}7 \times 10^3$ ct/min.

In vitro *antibody formation.* Humoral antibody responses to SRBC were evaluated by culturing 5×10^6 spleen cells with 2×10^6 SRBC in 2 ml of RPMI 1640 supplemented with 10% FCS, 5×10^{-5} M 2-mercaptoethanol, 100 units/ml penicillin, 100 μg/ml streptomycin, 1 mM glutamine, and 50 units/ml mycostatin in one well of a flat bottom (24 well) cluster plate (Costar). Cultures were

incubated for 5 days at 37°C in a humidified 5% CO_2 air atmosphere. The number of IgM plaque forming cells (PFC) per culture was determined by the Fauci & Pratt (1976) modification of the Jerne technique. Results are expressed as the mean PFC for triplicate cultures.

Statistical analysis. The level of significance for spleen cell responses was determined by the Student's *t*-test.

RESULTS

Splenic lymphocyte responses to heat killed H. capsulatum

Blastogenic responses to 5×10^5 HK-Hc were measured at different time intervals for control (normal) animals, and mice inoculated i.t. or i.v. with 5×10^5 live yeast cells of *H. capsulatum*. As

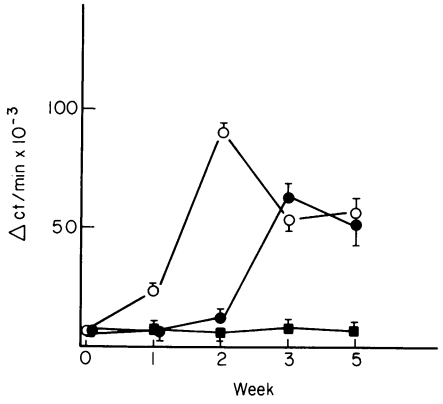

Fig. 1. Blastogenic responses to HK-Hc for splenocytes obtained from control (■) C57Bl/6 mice and mice inoculated with 5×10^5 viable yeast cells i.v. (●) or i.t. (○) at different time (weeks) intervals following infection. Each point represents the mean ct/min observed for experimental minus control ±1 s.d.

shown in Fig. 1, significant ($P < 0{\cdot}005$) levels of blastogenesis to HK-Hc were detected in splenocytes 1 week after i.t. inoculation. Responses to HK-Hc reached a maximum 2 weeks following i.t. infection and continued at a significant ($P < 0{\cdot}005$) level over the entire period of study, 5 weeks. In contrast, no significant blastogenesis to HK-Hc in spleen cells from i.v. inoculated animals was observed up to 2 weeks following infection. At 3 weeks, however, splenic responses to HK-Hc were comparable for either i.v. or i.t. inoculated animals and considerably ($P < 0{\cdot}005$) higher than responses obtained from normal (control) animals. The marked depression observed during the first 2 weeks of i.v. *H. capsulatum* infection was not related to assay conditions. Spleen cells from these animals were tested over a wide range of HK-Hc concentrations (1×10^3–5×10^6 yeast cells) and at different time intervals with results similar to those reported in Fig. 1. Additionally, splenocytes obtained from animals inoculated with HBSS alone yielded responses comparable to those obtained from unimmunized control animals.

Non-specific immune responses from H. capsulatum *inoculated mice*

In contrast to the previous findings, splenic alloantigen responses for i.t. inoculated animals were significantly ($P < 0{\cdot}005$) depressed 1 week following *H. capsulatum* infection (Fig. 2). However, this decrease in non-specific antigen reactivity was temporary since splenic MLR activity returned to normal levels by the 2nd week of infection. MLR responses from i.v. inoculated animals were also dramatically ($P < 0{\cdot}005$) lower than control responses 1 week following infection. Indeed, the immune depression at 1 week with i.v. inoculated animals was even more severe than that noted with i.t. inoculated mice ($P < 0{\cdot}05$). Continued study over several time intervals has shown that recovery of splenic MLR reactivity for i.v. inoculated mice occurs approximately 3 weeks following infection.

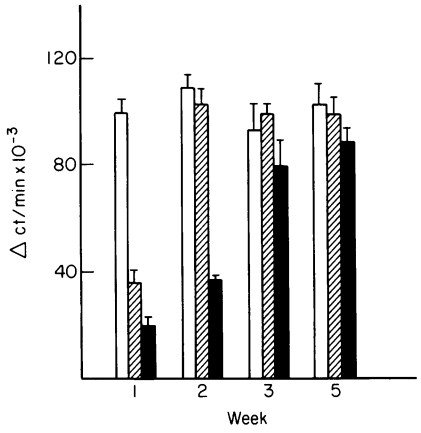

Fig. 2. Mixed lymphocyte responses by splenocytes obtained from age matched control (□) mice and mice inoculated i.v. (■) or i.t. (▨) with 5×10^5 *H. capsulatum* yeast cells at different times (weeks) following infection. Each bar represents the mean ct/min obtained from experimental minus control ± 1 s.d.

The antibody producing capacity of splenocytes from i.t. inoculated animals was significantly ($P < 0{\cdot}001$) lower than *in vitro* responses observed from control animals to SRBC (Fig. 3). The depression in antibody formation noted for i.t. inoculated mice was also transient with humoral responses returning to control levels by the 2nd week of *H. capsulatum* infection. On the other hand, splenocytes from i.v. inoculated mice exhibited a significant ($P < 0{\cdot}01$) and long lasting depression in antibody responses to SRBC. Complete recovery of the antibody-forming abilities was not apparent until 5 weeks after i.v. *H. capsulatum* infection. The lowered immune potential demonstrated by splenocytes from either i.t. (1 week) or i.v. (1, 2 and 3 weeks) inoculated mice was not related to antigen (SRBC) concentration since responses measured at several different SRBC concentrations revealed similar alterations in humoral immunity.

The influence of splenocytes from H. capsulatum *inoculated mice on normal spleen cell responses*

To determine whether the depression observed after i.t. *H. capsulatum* was associated with the generation of supressor cells, the influence of splenocytes from i.t. inoculated mice on the antibody forming capacity of normal splenocytes was examined. As shown in Table 1, *in vitro* antibody

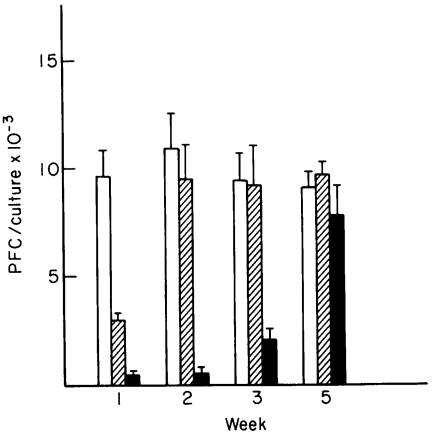

Fig. 3. Non-specific antibody responses to SRBC for spleen cells from age matched control (□) mice and mice inoculated iv. (■) or i.t. (▨) with 5×10^5 viable yeast cells. Each bar represents the mean number of PFC/culture ± 1 s.d.

Table 1. Primary antibody responses to SRBC by spleen cells 1 week following inoculation with 5×10^5 *H. capsulatum* yeast cells

Spleen cell cultures	Mean PFC/culture ±1 s.d.		
	Expt 1	Expt 2	Expt 3
Normal*	10,800±1,552	11,600±2,211	9,700±656
i.t. inoculated	2,100±529‡	2,800±485§	2,667±1,069§
Normal+i.t. inoculated†	8,900±721	8,667±709	10,400±700
i.v. inoculated	567±153‡	633±404§	1,833±208‡
Normal +i.v. inoculated	1,467±306§	1,700±818§	3,433±451‡

* $7{\cdot}5 \times 10^6$ spleen cells per culture.

† 5×10^6 normal spleen cells+$2{\cdot}5 \times 10^6$ spleen cells from i.v. or i.t. inoculated mice per culture.

‡ $P < 0{\cdot}005$.

§ $P < 0{\cdot}01$.

responses from cultures composed of a mixture of spleen cells from i.t. inoculated animals (1 week) and normal splenocytes yielded PFC responses similar to normal splenocytes alone. We have also determined that the depression in MLR responses is not linked to suppressor cell development in i.t. inoculated animals (data not shown). In contrast to these observations and as previously reported (Nickerson *et al.*, 1981; Watson & Bullock, 1982) the immune depression noted after i.v. infection is mediated through the generation of potent ($P < 0{\cdot}005$) suppressor cells (Table 1). The suppressor cell activity associated with i.v. inoculation persisted until approximately 3 weeks after *H. capsulatum* infection.

i.t. H. capsulatum *inoculum size and immune responsiveness*

The immune potential of animals receiving varying numbers of yeast cells was also evaluated to determine whether the non-specific immune depression observed during i.t. infection was related to inoculum size. One week following yeast cell inoculation, decreases in antibody production to SRBC were only noted for animals receiving i.t. inocula greater than or equal to 5×10^4 yeast cells (Table 2). i.t. inoculated animals were also tested at several different time periods, 2 and 3 weeks, after yeast cell exposure. At those times, antibody responses similar to control animals (normal or HBSS inoculated) were noted for i.t. inoculated mice except in those animals receiving $2{\cdot}5 \times 10^6$

Table 2. Effect of *H. capsulatum* inoculum size on primary antibody formation to SRBC

Inoculum size*	Mean PFC/culture ±1 s.d.	
	Expt 1	Expt 2
0	10,733±1,077	8,100±1,473
5×10^3	10,300±985	9,300±1,308
5×10^4	5,367±777†	8,533±1,193
5×10^5	1,867±751‡	1,533±551‡
$2{\cdot}5 \times 10^6$	2,133±153‡	—§

* Number of yeast cells administered i.t. 1 week prior to *in vitro* antibody assay.

† $P < 0{\cdot}05$.

‡ $P < 0{\cdot}01$.

§ Not tested.

yeast cells which appeared to require a longer recovery time, 2 weeks. In comparison to these findings, markedly impaired antibody production to SRBC was observed at 1 week in animals receiving the smallest inoculum (5×10^3 yeast cells) i.v., $2{,}067 \pm 987$ (mean PFC + 1 s.d.) when compared to responses obtained with normal spleen cells ($8{,}400 \pm 265$) or i.t. inoculated animals ($7{,}700 \pm 1{,}311$).

DISCUSSION

Our results show that significant numbers of antigen reactive cells to HK-Hc are generated in the spleen of C57Bl/6 mice shortly (1 week) after i.t. inoculation of *H. capsulatum* yeast cells. Tewari *et al.* (1982) have reported similar findings with different antigens (histoplasmin or ribosomes) following s.c. inoculation with *H. capsulatum* yeast cells. In contrast, blastogenic responses to HK-Hc were not detected until 3 weeks after i.v. inoculation with yeast cells. The time sequence required for the development of antigen specific spleen cell responses in i.v. inoculated animals resembles previous findings by Artz & Bullock (1979b) in C3H/ANF mice.

The apparent differences in the development of specific immunity following either i.t., s.c. or i.v. *H. capsulatum* infection does not seem to be related to the test antigen used, i.e. soluble or whole cells (Alford & Cartwright, 1981) although responses to whole yeast cells would probably better approximate *in vivo* reactivity. The delayed appearance of antigen specific spleen cell responses during i.v. infection may well be related to the development of splenic immunosuppressor cells in these animals. It is clear from previous work (Nickerson *et al.*, 1981) that several populations of suppressor cells, T lymphocytes and macrophage like cells, are generated following i.v. *H. capsulatum* infection. Furthermore, results in the present study and others (Artz & Bullock, 1979b; Nickerson *et al.*, 1981) demonstrate that the development of HK-Hc reactivity following i.v. infection corresponds to losses or significant decreases in splenic suppressor cell activity.

Examination of immune responses to alloantigens or SRBC has revealed that non-specific immune depression accompanies early i.t. infection even though specific immune responses to HK-Hc were clearly evident at that time. A similar paradox between specific and non-specific immunity has been described during s.c. *H. capsulatum* (Tewari *et al.*, 1982), *Leishmania mexicana* (Perez, Marisol & Malave, 1981), and *Trypanosoma brucei* (Hudson & Terry, 1979) infections. Although the precise mechanism(s) for the transient non-specific immune depression during i.t. infection remains to be defined, it is evident that suppressor cells were not associated with the decreases observed following sublethal pulmonary exposure to *H. capsulatum* (Table 2). It is important to note however, that this non-specific immune depression coincides with the time of highest antigen (*H. capsulatum*) load in i.t. inoculated animals (Nickerson, Fairclough & Sumner, unpublished observation). This information might suggest a role for antigenic competition in the observed immune depression. Furthermore, a large body of information supports the concept of antigenic competition during infection (Miller, Good & Mishell, 1978; Mitchell & Handman, 1977; Watson, Slijivic & Brown, 1975). Several studies (Mitchell & Handman, 1977; Watson *et al.*, 1975) have provided direct evidence for macrophage involvement in antigenic competition. It has been suggested that active infection, particularly intracellular infections, may interfer with the ability of macrophages to process other antigens such as SRBC (Miller *et al.*, 1978; Warren & Weidanz, 1976; Watson *et al.*, 1975). Although *H. capsulatum* is a facultative intracellular parasite (Howard, 1965), further work is required to determine the precise mechanism(s) responsible for the non-specific immune depression associated with i.t. infection.

The relative importance of antigenic exposure through a particular route has been known for many years to influence the ultimate outcome of an immune response, i.e. positive or negative. Recently, the positive or negative influences mediated by different routes of antigen administration have been directly related to the generation of helper or suppressor cells for the antigen under investigation (Greene, Sugimoto & Benecerraf, 1978; Richman *et al.*, 1978). This point is well illustrated in the present study since i.t. inoculation with *H. capsulatum* led to the development of specific immune responses while i.v. inoculation led to consistent and potent immune suppression. Considering this information, murine i.t. immunization would provide an excellent system for

determining the factor(s) responsible for immunosuppression in some, but not all, patients with chronic pulmonary or disseminated histoplasmosis.

Most human infections with *H. capsulatum* are self-resolving pulmonary diseases which lead to the development of specific immunity as evidenced by *in vivo* delayed type hypersensitivity (Alford & Goodwin, 1972; Goodwin & Des Prez, 1978) and *in vitro* blastogenic responses (Alford & Cartwright, 1981; Cox, 1979) to *H. capsulatum*. Furthermore, systemic immunity to *H. capsulatum* has been reported following experimental pulmonary exposure to *H. capsulatum* induced by aersols (Schlitzer, Chandler & Larsh, 1982) or i.t. inoculation (Bauman & Chick, 1973) of several species, i.e. guinea pigs and *Rhesus* monkeys. Our results suggest that i.t. yeast cell inoculation in mice would also represent a useful model for studies of at least systemic and perhaps in the future, pulmonary immunity to *H. capsulatum*.

This investigation was supported by U.S. Public Health Service grant AI-17750 from the National Institute of Allergy and Infectious Diseases.

REFERENCES

ALFORD, R.H. & CARTWRIGHT, B.B. (1981) Human lymphocyte blastogenesis induced by living and dead *Histoplasma capsulatum* yeasts and soluble yeast autolysate. *Sabouraudia*, **19,** 85.

ALFORD, R.H. & GOODWIN, R.A. (1972) Patterns of immune response in chronic pulmonary histoplasmosis. *J. Infect. Dis.* **125,** 269.

ARTZ, R.P. & BULLOCK, W.E. (1979a) Immunoregulatory responses in experimental disseminated histoplasmosis: lymphoid organ histopathology and serological studies. *Infect. Immun.* **23,** 884.

ARTZ, R.P. & BULLOCK, W.E. (1979b) Immunoregulatory responses in experimental disseminated histoplasmosis: depression of T-cell dependent and T-effector responses by activation of splenic suppressor cells. *Infect. Immun.* **23,** 893.

BAUMAN, D.S. & CHICK, E.W. (1973) Acute cavitary histoplasmosis in *Rhesus* monkeys: influence of immunological status. *Infect. Immun.* **8,** 245.

COX, R.A. (1979) Immunologic studies of patients with histoplasmosis. *Am. Rev. Resp. Dis.* **120,** 143.

FAUCI, A.S. & PRATT, K.R. (1976) Polyclonal activation of bone marrow-derived lymphocytes from human peripheral blood measured by a direct plaque-forming cell assay. *Proc. Natl. Acad. Sci. USA.* **73,** 3676.

GOODWIN, R.A. & DES PREZ, R.M. (1978) Histoplasmosis. *Am. Rev. Resp. Dis.* **117,** 929.

GREENE, M.I., SUGIMOTO, M. & BENACERRAF, B. (1978) Mechanisms of regulation of cell-mediated immune responses. I. Effect of route of immunization with TNP-coupled syngenic cells on the induction and supression of contact sensitivity to picryl chloride. *J. Immunol.* **120,** 1604.

HOWARD, D.H. (1965) Intracellular growth of *Histoplasma capsulatum*. *J. Bacteriol.* **93,** 438.

HUDSON, K.M. & TERRY, R.J. (1979) Immunodepression and the course of infection of a chronic *Trypanosoma brucei* infection in mice. *Parasite Immunol.* **1,** 317.

MILLER, K.L., GOOD, A.H. & MISHELL, R.I. (1978) Immunodepression in *Taenia crassiceps* infection: restoration of the *in vitro* responses to sheep erythrocytes by activated peritoneal cells. *Infect. Immun.* **22,** 365.

MITCHELL, G.F. & HANDMAN, E. (1977) Studies on immune responses to larval cestodes in mice: a simple mechanism of non-specific immunosupression in *Mesocestoides corti*-infected mice. *Aust. J. exp. biol. Med. Sci.* **55,** 615.

NEWBERRY, W.M., CHANDLER, J.M., CHIN, T.D.Y. & KIRKPATRICK, C.H. (1968) Immunology of the mycoses. I. Depressed lymphocyte transformation in chronic histoplasmosis. *J. Immunol.* **100,** 436.

NICKERSON, D.A., HAVENS, R.A. & BULLOCK, W.E. (1981) Immunoregulation in disseminated histoplasmosis: characterization of splenic suppressor cell populations. *Cell. Immunol.* **60,** 287.

PEREZ, H., MARISOL, P. & MALAVE, I. (1981) Nonspecific immunodepression and protective immunity in mice infected with *Leishmania mexicana*. *Infect. Immun.* **32,** 415.

REDDY P., GORELICK, D.F., BRASHER, C.A. & LARSH, H. (1976) Progressive disseminated histoplasmosis as seen in adults. *Am. J. Med.* **48,** 629.

RICHMAN, L.K., CHILLER, J.M., BROWN, W.R., HANSON, D.G. & YAZ, N.M. (1978) Enterically induced immunologic tolerance. I. Induction of suppressor T lymphocytes by intragastric administration of soluble antigens. *J. Immunol.* **121,** 2429.

SCHLITZER, R.L., CHANDLER, F.W. & LARSH, H.W. (1982) Primary acute histoplasmosis in guinea pigs exposed to aerosolized *Histoplasma capsulatum*. *Infect. Immun.* **33,** 575.

SIMPSON, E., GORDON, R., TAYLOR, M., MERTIN, J. & CHANDLER, P. (1975) Micromethods for induction and assay of mouse mixed lymphocyte reactions and cytotoxicity. *Eur. J. Immunol.* **5,** 451.

SMITH, J.W. & UTZ, J.P. (1972) Progressive disseminated histoplasmosis. A prospective study of 26 patients. *Ann. Intern. Med.* **76,** 557.

STOBO, J.D., SIGRUN, P., VANSCOY, R.E. & HERMANS, P.E. (1976) Suppressor thymus-derived lymphocytes in fungal infections. *J. clin. Invest.* **57,** 319.

TEWARI, R.P., KHARDORI, N., MCCONNACHIE, P., VONBEHREN, L.A. & YAMADA, T. (1982) Blastogenic responses of lymphocytes from mice immunized by sublethal infection with yeast cells of *Histoplasma capsulatum*. *Infect. Immun.* **36,** 1013.

WARREN, H.S. & WEIDANZ, W.P. (1976) Malarial

immunodepression *in vitro*: adherent spleen cells are functionally defective as accessory cells in the responses to horse erythrocytes. *Eur. J. Immunol.* **6,** 816.

WATSON, S.R. & BULLOCK, W.E. (1982) Immunoregulation in disseminated histoplasmosis: characterization of the surface phenotype of splenic suppressor T lymphocytes. *Infect. Immun.* **37,** 940.

WATSON, S.R., SLJIVIC, V.S. & BROWN, I.N. (1975) Defect of macrophage function in the antibody response to sheep erythrocytes in systemic *Mycobacterium lepraemurium* infection. *Nature*, **256,** 206.