effective immunogens unless administered along with a powerful adjuvant. Based on these findings, it is very unlikely that the anti-ZP antibodies found in the serum of the human female are a normal physiological consequence of isoimmunization to her own ovulated zona components.

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1 Dunbar, B. S., in: Mechanism and Control of Fertilization, p. 139. Ed. J. Hartmann. Academic Press, New York 1983.
2 Dunbar, B. S., in: Immunology of Reproduction, p. 505. Eds T. Wegmann and T. Gill. Oxford University Press, New York 1983.
3 Skinner, S. M., and Dunbar, B. S., in: Immunological Approaches to Contraception and Promotion of Fertility, p. 251. Ed. G. P. Talwar. Plenum Press, New York 1986.
4 Wood, D. M., and Dunbar, B. S., J. exp. Zool. *217* (1981) 423.
5 Shivers, C. A., and Dunbar, B. S., Science *197* (1977) 1187.
6 Tsunoda, Y., and Chang, M. C., Biol. Reprod. *20* (1979) 355.
7 Trounson, A. D., Shivers, C. A., McMaster, R., and Lapata, A., Archs Androl. *4* (1980) 29.
8 Mhaskar, A., Buckshee, K., and Talwar, G. P., Contraception *29* (1984) 75.
9 Sacco, A. G., and Moghissi, K. S., Fert. Steril. *31* (1979) 503.
10 Dunbar, B. S., Wardrip, N. J., and Hedrick, J. L., Biochemistry *19* (1980) 356.
11 Sehgal, S., Gupta, S. K., and Bhatnagar, P., Pathology (1989) in press.
12 Voller, A., Bartlett, A., and Bidwell, D. E., J. clin. Path. *31* (1978) 507.
13 Dunbar, B. S., Liu, C., and Sammons, D. W., Biol. Reprod. *24* (1981) 1111.
14 Dunbar, B. S., in: Two-dimensional Gel Electrophoresis and Immunological Techniques. Plenum Press, New York 1987.
15 Sammons, D. W., Anderson, L. D., and Nishiawa, E. E., Electrophoresis *2* (1981) 135.
16 Sammons, D. W., Adams, L. D., Vidmar, T. J., Hatfield, C. A., Jones, U. H., Chuba, P. J., and Crooks, S. W., in: Two-dimensional Gel Electrophoresis of Proteins: Methods and Applications, p. 112. Eds J. E. Celis and R. Bravo. Academic Press, New York 1984.
17 Timmons, T. M., Maresh, G. A., Bundman, D. S., and Dunbar, B. S., Biol. Reprod. *36* (1987) 1275.
18 Hedrick, J. L., and Wardrip, N. J., Analyt. Biochem. *157* (1986) 63.
19 Yurewicz, E. C., Sacco, A. G., and Subramanian, M. G., J. biol. Chem. *262* (1987) 564.
20 Skinner, S. M., Mills, T., Kirchick, H. J., and Dunbar, B. S., Endocrinology *115* (1984) 2418.
21 Sacco, A. G., Subramanian, M. C., Yurewicz, E. C., deMayo, F. J., and Dukelow, W. R., Fert. Steril. *39* (1983) 350.
22 Sacco, A. G., Biol. Reprod. *16* (1977) 164.
23 Sacco, A. G., and Palm, V. S., J. Reprod. Fert. *51* (1977) 165.
24 Sacco, A. G., Yurewicz, E. C., Subramanian, M. G., and deMayo, F. J., Biol. Reprod. *25* (1981) 997.

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## Suppression of phytohemagglutinin induced splenocyte proliferation during concurrent infection with *Eimeria nieschulzi* and *Nippostrongylus brasiliensis*[1]

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*Summary.* Results suggest that infection with *Eimeria nieschulzi* (Protozoa) interferes with splenocyte proliferation induced by infection with *Nippostrongylus brasiliensis* (Nematoda).
*Key words. Eimeria nieschulzi; Nippostrongylus brasiliensis*; splenocyte; blastogenesis; PHA.

Numerous studies have demonstrated that some parasites can alter immune function in infected hosts[3]. However, with the exception of *Toxoplasma gondii*[4] few studies have been published demonstrating depression of mitogen-induced lymphocyte blastogenesis by coccidia. Recently, however, Rose and Hesketh[5] showed that serum from *Eimeria tenella* infected chickens is capable of depressing mitogen induced T- and B-lymphocyte blastogenesis in vitro. Since previous research has demonstrated delayed expulsion of helminths during concurrent infections with *Eimeria nieschulzi* and *Nippostrongylus brasiliensis*[6,7] as well as decreased numbers of intestinal polymorphonuclear leukocytes, suppressed systemic granuloma formation, and a suppression of eosinophil release from the bone marrow during *E. nieschulzi* infections[7,8] it was of interest to us to explore whether serum from rats infected with *E. nieschulzi* is capable of altering splenocyte blastogenesis since these cells are involved in immune regulation.

*Materials and methods*

Forty specific-pathogen-free male TEX: (SD) AM Sprague-Dawley outbred rats (Harlan Breeding Laboratories, Houston, TX), weighing 200–250 g each, were housed in autoclaved cages with sterile wood shavings for bedding. Rats were given commercial rodent chow and water ad libitum, and kept on a 12-h light/dark cycle. After allowing the animals to acclimate to the cages for 2 weeks, feces were examined for extraneous protozoa and helminth ova by flotation[6]. Rats were then divided on day 0 into 4 groups of 10 rats each. Group 1 served as uninfected controls. Group 2 rats were each inoculated per os with $2.5 \times 10^5$ sporulated oocysts of *E. nieschulzi* and sacrificed on day 8 postinoculation (PI). Rats in group 3 were each administered subcutaneously 2000 $L_3$ larvae of *N. brasiliensis* and sacrificed on day 16 PI. Group 4 animals were inoculated with *N. brasiliensis* larvae and on day 8 PI, *E. nieschulzi* was administered; rats were sacrificed on day 16 PI of the helminth infec-

tion. Flotations were performed on intestinal contents of each animal at the time of sacrifice and examined microscopically to again ensure the absence of extraneous intestinal parasites and the presence of patent *N. brasiliensis* and *E. nieschulzi* infections. Rats were etherized, blood was removed from each animal by cardiac puncture and allowed to clot overnight at 4 °C. After centrifugation of the blood, serum was removed, pooled, divided into equal aliquots, and stored at − 70 °C until use. Other tissues were removed from all groups after blood was obtained and processed for various studies. Results of these investigations were published by Upton et al.[7].

Blastogenesis assays were replicated three times using pooled sera from each of the 4 groups. Single cell suspensions were prepared from uninfected rats by teasing spleens through stainless steel mesh in RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 1 mM L-glutamine, 100 IU/ml penicillin, and 100 µg/ml streptomycin. Splenocytes from a single rat were used for each replicate assay. Erythrocytes were lysed by placing the spleen suspension in ammonium chloride-Tris HCl, pH 7.3, for 5 min at 37 °C. Cells were cultured in RPMI 1640 with supplements at 37 °C in 5% $CO_2$/95% air in flat-bottom microtiter plates at a concentration of $1 \times 10^6$ cells/well; wells contained 3% rat serum (non-heated or heat inactivated at 56 °C for 30 min) from one of the 4 groups and ± 1.0 µg phytohemagglutinin (PHA) (*Phaseolus vulgaris*; Sigma Chemical Company, St. Louis, MO) in a total volume of 200 µl. At 52 h, 0.5 µCi $^3$H-thymidine was added to each well and cultures reincubated. Cells were harvested 18 h later onto microfiber filters, dried for 30 min at 50 °C, and the fiber discs placed in scintillation vials with 5.0 ml Aquasol-2 (New England Nuclear, Boston, MA). Radioactivity was measured with a Beckman LS 230 scintillation counter. Data were expressed as counts/min and the replicates pooled. Results were then expressed as the stimulation index (SI) obtained as follows: Mean counts/minute (cpm) of stimulated test culture/mean cpm of unstimulated test culture divided by the mean cpm of stimulated control culture/mean cpm of unstimulated control culture[5]. The unity value was set at 1.0 (no blastogenic effect) with SI values of > 2.0 considered to indicate enhanced stimulation of blastogenesis.

*Results and discussion*

No extraneous protozoa or helminths were found in any of the rats used in the present study, thus, the effects observed are attributable only to the presence of *N. brasiliensis* and/or *E. nieschulzi*. Results presented in the table illustrate that normal splenocytes cultured with PHA and non-heat inactivated sera from rats infected only with *N. brasiliensis* had a higher SI than splenocytes cultured in serum from either *E. nieschulzi* infected rats or rats harboring concurrent infections. This indicates that serum from rats infected with the helminth enhances the response to PHA. This effect proved to be heat labile.

Response to phytohemagglutinin by normal spleen cells cultured in sera collected from rats infected with *Eimeria nieschulzi* (EN) and/or *Nippostrongylus brasiliensis* (NB)

| Parasite* | Day postinoculation of sacrifice | Heat inactivation (±) | SI$^+$ |
|---|---|---|---|
| EN | 8 | − | 1.1 |
| | | + | 0.8 |
| NB | 16 | − | 2.1 |
| | | + | 0.6 |
| EN/NB | 8/16 | − | 0.8 |
| | | + | 0.7 |

* N = 10 rats/group; sera pooled. $^+$ Stimulation index; values are means obtained from three replicate blastogenesis assays.

Although the mechanism by which this phenomenon occurs is unknown, it is not unexpected, considering the degree of immunostimulation known to occur during *N. brasiliensis* infections[9]. Stockinger and Konig[10], however, demonstrated suppression of T-lymphocyte blastogenesis using PHA and serum from a different strain of *N. brasiliensis* infected rats.

Splenocytes cultured with PHA and sera from rats infected singly with *E. nieschulzi*, or concurrently with the coccidian and helminth, had SI values near unity indicating a lack of the splenocyte response to PHA; these SI values were not different from those obtained when the serum was heated to abolish the blastogenic effect. Thus, it can be inferred that infection with *E. nieschulzi* interferes with the stimulatory effect of *N. brasiliensis* infection. This is the first report of this phenomenon being induced by a rodent coccidian, however, a similar response has been observed for normal chicken lymphocytes cultured in plasma from infected chickens[5]. The mechanism by which the coccidia modulate mitogen induced splenocyte proliferation is unknown, however, it is clear that a serum factor(s) is (are) involved. Whether they are derived from the host or parasite is also unknown. A variety of serum soluble immunosuppressive factors have been demonstrated in parasitic infections, including immune complexes of *Plasmodium chabaudi*[11–13] and (apparently) host generated suppressor substances in *T. gondii* and *Trypanosoma cruzi* infections[4, 14]. Whether or not the mechanism of *Eimeria* induced immune suppression is similar is unknown.

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2 To whom all correspondence should be addressed.
3 Terry, R. J., and Hudson, K. M., Fortsch. Zool. *27* (1982) 125.
4 Chan, J., Siegel, J. P., and Luft, B. J., Cell. Immun. *98* (1986) 422.
5 Rose, M. E., and Hesketh, P., J. Protozool. *31* (1984) 549.
6 Bristol, J. R., Piñon, A. J., and Mayberry, L. F., J. Parasit. *69* (1983) 327.
7 Upton, S. J., Mayberry, L. F., Bristol, J. R., Favela, S. H., and Sambrano, G. R., J. Parasit. *73* (1987) 300.
8 Castro, G. A., and Duszynski, D. W., J. Protozool. *31* (1984) 283.
9 Kassai, T., Handbook of *Nippostrongylus brasiliensis* (Nematoda). Commonwealth Agricultural Bureau, Akademiai Kiado, Budapest 1982.
10 Stockinger, H., and Konig, W., Adv. exp. Med. Biol. *116* (1979) 693.
11 Cox, H. W., Hayes, M. M., and Saleh, S. M., J. Parasit. *69* (1983) 809.
12 Degraves, F. J., and Cox, H. W., J. Parasit. *69* (1983) 262.
13 Cox, H. W., and Hayes, M. M., J. Parasit. *71* (1985) 50.
14 Cunningham, D. S., and Kuhn, R. E., J. Parasit. *66* (1980) 16.

0014-4754/89/080762-02$1.50 + 0.20/0