

EIMERIA COLCHICI INFECTION IN CHICKENS: THE INVASION AND LOCAL IMMUNE RESPONSE OF A NON-SPECIFIC HOST

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The local immune response of chickens, as a non-specific host to, experimental infection with Eimeria colchici, coccidia from pheasants, was studied. The pheasant Eimeria invaded the intestine of the chickens not only in the caecum, as is the case in the natural host, but also in the cranial part of the intestine (duodenum). In these two infected areas we subsequently counted the number of CD3 positive T lymphocytes and eosinophilic granulocytes (heterophils and eosinophils). The numbers of eosinophilic granulocytes gradually increased during the infection period. Immunohistochemical staining on paraffin sections was used to characterize CD3 positive lymphocytes. We observed a marked increase in CD3 positive cells from 36 hours post infection onwards. In conclusion, the significant increase of CD3 positive lymphocytes after the infection suggests that these cells are involved in the induction of the immune response and might prevent the further development of Eimeria in this non-specific host.

Key words: chicken, E. colchici, eosinophils, heterophils, IELs, lamina propria, T lymphocytes

INTRODUCTION

Infection with any one of the *Eimeria* spp. that parasitise fowl induces resistance to reinfection, manifest by diminution of clinical and pathological effects and replication of the parasite. Host immunity is species specific, although a little cross-protection is observed against heterologous species and, in some cases, against different strains of the same species (Martin *et al.* 1997). Augustine and Danforth (1990) have shown that chickens repeatedly inoculated with *Eimeria adenoides* develop a measure of immunity, which protects them at least partially from a subsequent moderate challenge with *Eimeria tenella*. We have previously shown the invasion of *E. colchici* in the chicken caecum and non-specific localisation in the small intestine. The number of *E. colchici* schizonts in Leghorn chick caeca was significantly lower than numbers in the caeca of pheasant chicks infected with *E. colchici* evaluated at a similar time post infection. The localisation and comparison of the schizonts size revealed some differences between the specific and non-specific hosts. The general impact of infection on the systemic immune system was indicated by an increase in the number of lymphocytes and

their subpopulations (Looszová et al., in press). The mechanisms preventing the intracellular development of *Eimeria* in non-specific hosts are not fully understood. Cell-mediated immunity has been shown to have a dominant role in the host-protective response to *Eimeria* infection (Rose and Hesketh, 1982; Wakelin and Rose, 1990; Lillehoj and Trout, 1993). Intraepithelial (IEL) and lamina propria leucocytes (LPL) are the first line of defence in the intestine. Subpopulations of lymphocytes can be studied by using monoclonal antibodies (MoAb) and polyclonal antibodies (PoAbs) included in CD system (cluster of differentiation) of systematic nomenclature (Levkutova and Levkut, 1992; Levkut et al., 1994). Immunohistochemical techniques have shown that CD3 cells are present both in the epithelium and lamina propria of the chicken intestine (Lillehoj and Chung, 1992). At present, few antibodies can be used in formalin-fixed and paraffin-embedded tissue. A CD3 polyclonal antibody is commercially available and has been successfully used under different pathological conditions in several animal species (Ramos-Vara et al., 1994; Levkut et al., 1995; Sevcikova, 1997).

The purpose of our study was to follow the changes of CD3 positive T lymphocytes, eosinophilic granulocytes (heterophils and eosinophils) at the site of *E. colchici* invasion and possible development of the parasite in the intestine of a non-specific host.

MATERIAL AND METHODS

Experimental design. Thirty-six White Leghorn chicks were raised in standard poultry cages with free access to non-medicated food and water. At 10 days of age, the birds were divided into treatment and control groups. The pure culture of *Eimeria colchici* was obtained by isolating single oocysts on agar (Tsutsumi, 1972). The treatment group were orally inoculated with 10^6 oocysts per bird, while the control group was infected with a "placebo" of inoculum buffer only. Intestinal samples were taken from the infected and control birds 12, 36 and 60 hours post infection (h.p.i.).

Histological processing. For preparation of semi-thin sections the intestine was immediately immersed in a fixative solution consisting of a mixture of 2.5% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.2). Samples were postfixed in 0.1% OsO₄, dehydrated by increasing ethanol contrations, and embedded in Durcupan. Semi-thin sections 1-2 μ m thick were made on a Pyramitom (LKB), stained with 0.5% toluidine blue, and then evaluated under a light microscope. The tissue samples were fixed in 10% neutral formalin and subjected to routine processing. Another set of 6 μ m thick histological sections were cut and stained with haematoxylin-eosin.

Immunohistochemical procedure. Immunohistochemical detection of CD3 positive T lymphocytes in paraffin sections was based on the method described by Sevcikova (1997). The slides were dewaxed in xylene (2x10 min.) 96% benzylalkohol (2x8 to 10 min.) and 70% ethyl alcohol (5 min.). After inhibition of the endogenous peroxidase activity in 3% H₂O₂, the sections were washed in distilled water and digested in 0.4 % pepsin in 0.01 N HCl at 37°C for 30 min. After being washed, the sections were incubated with rabbit anti-human T cell CD3 antibody (Dacopatts, Glostrup, Denmark) overnight at 4°C. After washing in PBS-Tris-HCl buffer, the slides were incubated with biotinylated antirabbit immunoglobulin at room temperature for 30 min and then with peroxidase

conjugated streptavidin (Biogenex Laboratories, San Ramon, California, USA). The reaction was developed using a diaminobenzidine (DAB) derivate and counterstained in Mayers haematoxylin.

The counting of cells. The number of eosinophilic granulocytes (heterophils and eosinophils) was counted in the epithelium and lamina propria mucosae of the duodenum and caecum in 100 fields ($625 \mu\text{m}^2$) under the light microscope.

The number of CD3-positive lymphocytes in the epithelium (intraepithelial lymphocytes - IEL) and in the lamina propria mucosae (lamina propria lymphocytes - LPL) of the duodenum and caecum were counted by light microscopy in 50 fields ($2425 \mu\text{m}^2$).

Statistical analysis. Results were expressed as the mean SD and evaluated by the two-tailed paired Students t-test. A confidence level of P.05 was considered significant.

RESULTS

Histological examination of the chicks intestine showed similar results to our previous study (Looszova et al., in press). First generation schizonts of *Eimeria colchici* were found in the epithelial cells of the intestine primarily in the duodenum and caecum (Figure 1). The parasite was much smaller and the development was delayed in the non-specific host.

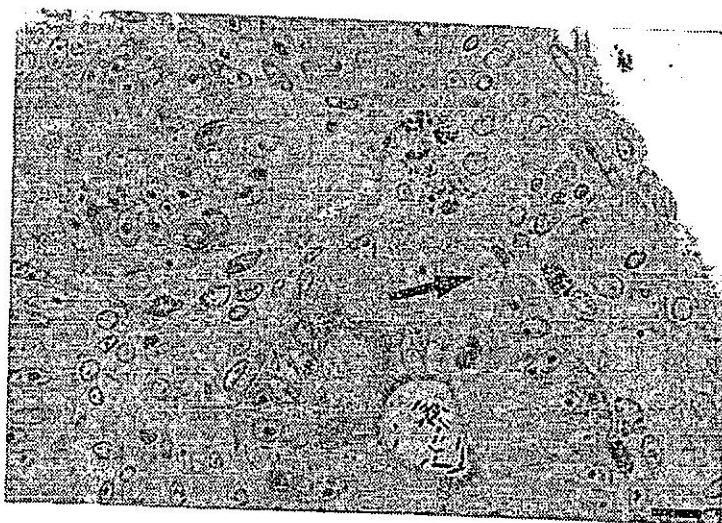


Fig. 1. First generation schizonts of *Eimeria colchici* (arrow) in epithelial cells of chicken caecum 12 hours post infection. Semi-thin section stained with toluidine blue. (bar = $1 \mu\text{m}$)

The number of eosinophilic granulocytes gradually increased as the infection developed in the duodenum and caecum of infected chickens (Fig. 4,5). Significant differences between the infected and control groups were found in both duodenum and caecum 60 hours post infection (h.p.i.) ($P < 0.05$).

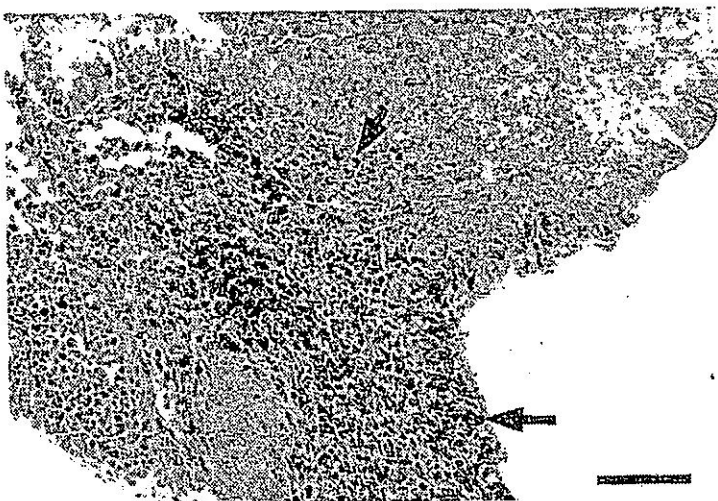


Fig. 2. CD3 cells (arrows) in the caecum of the experimental chickens 36 hours post infection with *Eimeria colchici* (bar = 5 μ m)

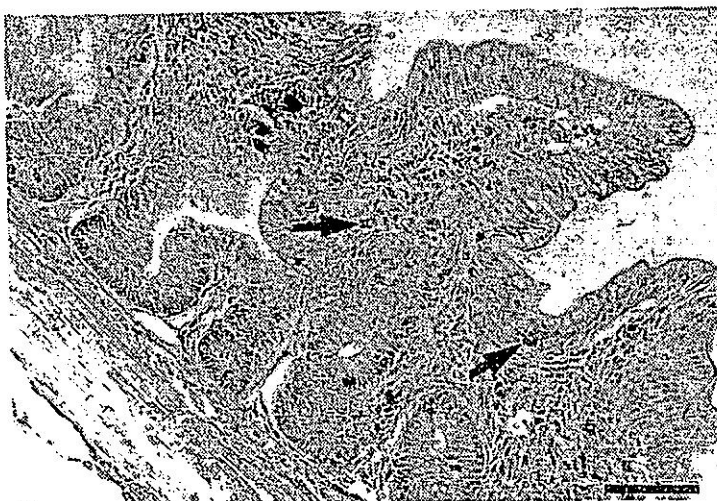


Fig. 3. CD3 cells (arrows) in the caecum of the control chickens (bar = 5 μ m)

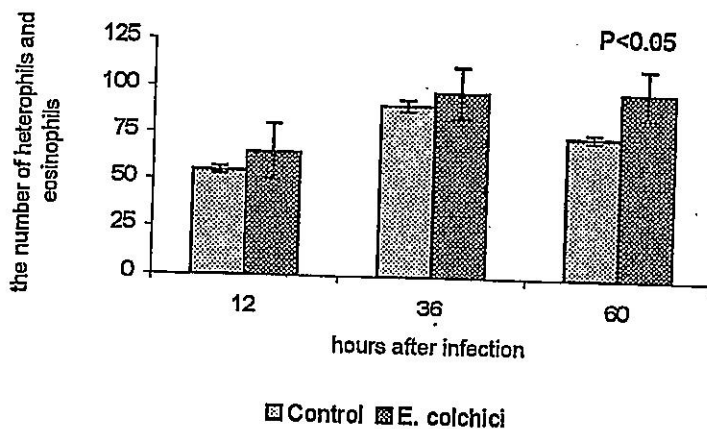


Fig. 4. The number of heterophils and eosinophils in the duodenum of Leghorn chicks after *Eimeria colchici* infection.

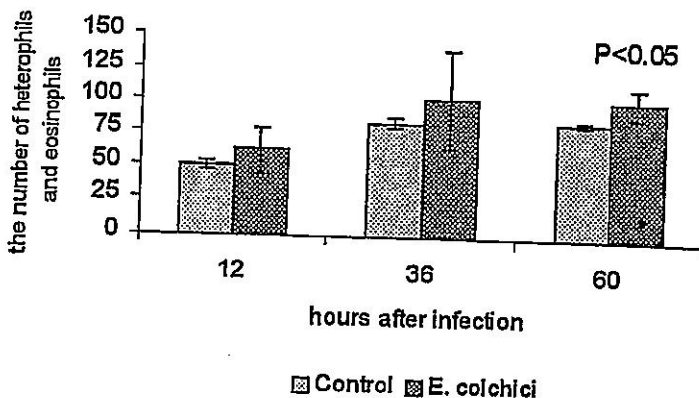


Fig. 5. The number of heterophils and eosinophils in the caecum of Leghorn chicks after *Eimeria colchici* infection.

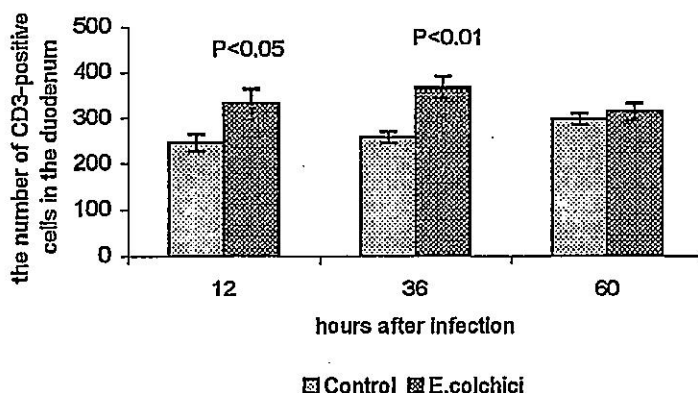


Fig. 6. The number of CD3-positive cells in the duodenum of Leghorn chicks after *Eimeria colchici* infection

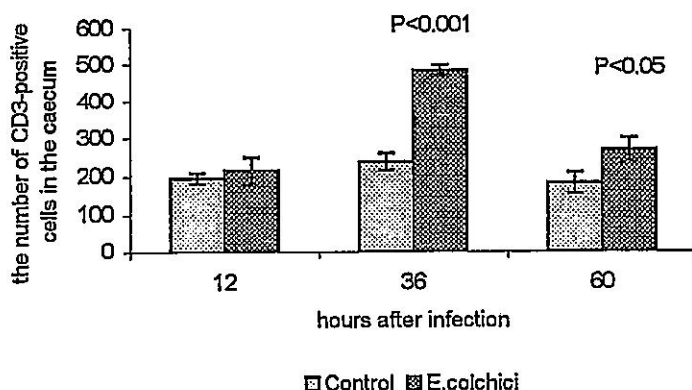


Fig. 7. The number of CD3-positive cells in the caecum of Leghorn chicks after *Eimeria colchici* infection

The effect of infection with *E.colchici* on the accumulation of CD3 positive intraepithelial and lamina propria T lymphocytes in the affected area of the intestine is illustrated in Figures 2 and 3. The duodenum, as a non-specific site of *E. colchici* infection in the natural host, showed a significant increase of CD3 T lymphocytes at 12 h.p.i. ($P < 0.05$) and 36 h.p.i. ($P < 0.01$) (Figure 6) in infected Leghorn chickens compared to the control group. The caecum, a specific site of *E. colchici* parasitism, showed a significant increase of CD3 positive intraepithelial and lamina propria T lymphocytes at 36 h.p.i. ($P < 0.001$). This increase was observed also at 60 h.p.i. ($P < 0.05$) (Figure 7).

DISCUSSION

Although most species of avian *Eimeria* exhibit marked host specificity for their development in vivo, they do not appear to exhibit the same degree of host specificity for invasion (Long and Millard, 1976; Vetterling, 1976). In the present study, we found that sporozoites of *E. colchici* invade approximately the same areas of the intestine in a foreign host as in the natural host, but not specifically the duodenum. Staining procedures using parasite specific monoclonal antibodies showed that sporozoites of turkey coccidia survived in the intestinal cells of chickens for up to 3 days but failed to develop any further (Augustine et al., 1991). Sporozoites of chicken coccidia also invaded the turkey, which is a foreign host, in the same intestinal sites as in the natural host (Augustine and Danforth, 1986), suggesting that sporozoites of chicken coccidia, like those of turkey coccidia, would be capable of eliciting cross-species protection.

In the duodenum and caecum of *E. colchici*-infected chickens we found a massive infiltration of eosinophilic granulocytes. Migration of heterophils to the intestine is a part of the host inflammatory response. Although heterophils might ingest and kill microorganisms Brune et al., (1972) and other investigators have found changes in the numbers of these cells in peripheral blood following an *Eimeria* infection, their role in the immune response is yet to be elucidated (Vervelde et al., 1996).

Parasite development in the chicken intestine is associated mainly with infiltration of lymphocytes (Lillehoj, 1998). Evaluation of CD3 positive cells in the intestinal mucosa during *E. colchici* infection in chickens demonstrated a significant increase of these cells in the affected areas. Analysis of CD3-positive cells in poultry shows that there are three mutually exclusive sublineages of CD3-positive cells recognised by TCR1 (Sowder et al., 1988), TCR2 (Cihak et al., 1988) and TCR3 (Char et al., 1989). The intestine is mostly infiltrated by TCR1+ and TCR2+ cells, which express CD4+ or CD8+ markers (Chen et al., 1988). The primary contact with invading sporozoites activates a cascade of defence mechanisms. Different leucocyte subpopulations are attracted to the inflammation site, where activated macrophages may modulate the severity of infection, whereas CD4 positive T cells may act as inducers of an effective immune response (Jeurissen et al., 1996).

The role of T lymphocytes on the development of turkey coccidia in chickens was investigated to document further the involvement of immune mechanisms in the reactions of non-specific hosts to infections with species of *Eimeria*. Kogut and Eirmann (1991) demonstrated that suppression of T lymphocyte activity in a non-specific host early during an infection with a heterologous species of *Eimeria* permits the complete intracellular development of the parasite. These findings are suggestive of a role for T lymphocytes in preventing the development of *Eimeria* in non-specific hosts possibly via a lymphokine-mediated mechanism.

Based on the results from this experiment, where the cellular events occurring during coccidial infection of non-specific hosts were examined, it can be concluded that CD3-positive T lymphocytes have a direct effect on preventing the development of the *Eimeria*. Upon the invasion of chicks by sporozoites of pheasant *Eimeria*, an inflammatory response was induced, resulting in T-cell activation and heterophil and eosinophil infiltration. Subsequent studies should be done for a better understanding of the immune mechanisms taking place in this non-specific host-parasite interaction.

REFERENCES

1. Augustine P.C, Danforth H.D., 1986, A study of dynamics of the invasion of immunized birds by *Eimeria* sporozoites. *Avian Dis*, 30, 347-351.
2. Augustine P.C, Danforth H.D., 1990, *Avian Eimeria*: invasion in foreign host bird and generation of partial immunity against coccidiosis. *Avian Dis*, 34, 196-220.
3. Augustine P.C, Danforth H.D., Barta J.R., 1991, Development of protective immunity against *Eimeria tenella* and *Eimeria acervulina* in White Leghorn chickens inoculated repeatedly with high doses of turkey coccidia. *Avian Dis*, 35, 535-541.
4. Brune K, Leffell M.S., Spitznagel J.K., 1972, Microbicidal activity of peroxidaseless chicken heterophile leucocytes. *Infect Immun*, 5, 283-287.
5. Char D, Chen C.H., Bucy R.P., Cooper M.D., 1989, Identification of a third T cell receptor (TCR3) in the chicken with a monoclonal antibody. *Fed Proc*, 3, 1507.
6. Chen C.H., Cihak J., Lösch U., Cooper M.D., 1989, Differential expression of two T cell receptors, TCR1 and TCR2, on chicken lymphocytes. *Eur J Immunol*, 18, 539-543.
7. Cihak J., Ziegler-Heitbrock H.W.L., Trainer H., Schraner I., Merckenschlager M., Lösch U., 1988, Characterization and functional properties of a novel monoclonal antibody which identifies a T-cell receptor in chicken. *Eur J Immunol*, 18, 535-537.
8. Kogut M.H., Eirmann L., 1991, The effect of cyclosporin A on the development of *Eimeria* in non-specific hosts. *Int J Parasitol*, 21, 979-983.
9. Levkutova M., Levkut M., 1992, Possibility of using leukocytic differential antigens in veterinary medicine. (In Slovak). *Veterinárstvi*, 42, 382-383.
10. Levkut M., Levkutova M., Rosival I., 1994, Leukozytäre Differenzierungsantigene in der Veterinärmedizin. *Wien Tier Monat*, 81, 117-121.
11. Levkut M., Kolodzieyski L., Ledecy V., Daxnerova K., 1995, Familial melanoma in the progeny of a Duroc and Slovak white crossbred sow. *J Comp Path*, 113, 233-239.
12. Lillehoj H.S., Chung, K., 1992, Postnatal development of T-lymphocyte subpopulations in the intestinal intra-epithelium and lamina propria in chickens. *Vet Immunol Immunopathol*, 31, 347-360.
13. Lillehoj H.S., Trout J.M., 1993, Coccidia: a review of recent advances on immunity and vaccine development. *Avian Pathol*, 22, 3-21.
14. Lillehoj H., 1998, Role of T lymphocytes and cytokines in coccidiosis. *Int J Parasitol*, 28, 1071-1081.
15. Long P.L., Millard V., 1976, Studies on the site finding and site specificity of *Eimeria praecox*, *Eimeria maxima*, *Eimeria acervulina* in chickens. *Parasitol*, 73, 327-336.
16. Looszova, A., Revajova, V., Levkut, M., Pistl, J., 2000, Immunological changes during *Eimeria colchici* infection in non-specific host. *Acta Vet. Brno*. In press.
17. Martin A.S., Danforth H.D., Barta J.R., Fernando M.A., 1997, Analysis of immunological cross-protection and sensitivities to anticoccidial drugs among five geographical and temporal strains of *Eimeria maxima*. *Int J Parasitol*, 27, 527-533.
18. Ramos-Vara J.A., Miller M.A., Lopez E., Brevik L., 1994, Reaction of polyclonal human CD3 antiserum in lymphoid tissue of cattle, sheep, goats, rats and mice. *Am J Vet Res*, 55, 63-66.
19. Rose M.E., Hesketh P., 1982, Immunity to coccidia in chickens: adoptive transfer with peripheral blood lymphocytes and spleen cells. *Parasite Immunol.*, 54, 171-185.
20. Sowder, J.T., Chen, C. H., Cihak, J., Lösch, U., Cooper, M. D., 1988, T-cell ontogeny: suppressive effects of embryonic treatment with monoclonal antibodies to T3, TCR1 or TCR2. *Fed Proc*, 2, 874.
21. Ševčíková Z., 1997, CD3 cells in the intestine of broilers inoculated with intestinal homogenate. *Br Poult Sci*, 38, 55-58.
22. Tsutsumi Y., 1972, *Eimeria tsunodai* sp. nov. (Protozoa: Eimeriidae) a caecal coccidium of Japanese quails (*Coturnix coturnix japonica*). *Jap J Vet Sci*, 34, 1-9.
23. Vervelde L., Vermeulen A.N., Jeurissen S.H., 1996, *In situ* characterization of leucocyte subpopulations after infection with *Eimeria tenella* in chickens. *Parasite Immunol.*, 18, 247-256.
24. Vetterling, J.M., 1976, *Eimeria tenella*: host specificity in gallinaceous birds. *J Protozol*, 23, 155-158.
25. Wakelin D., Rose M.E., 1990, Immunity to coccidiosis. In: *Coccidiosis of Man and Domestic Animals*. P. L. Long, ed. CRC Press, Boca Raton, Florida., 281-306.

INFEKCIJA PILIĆA SA *E. COLCHICI* - INVAZIVOST I LOKALNI IMUNOLOŠKI ODGOVOR KOD NESPECIFIČNOG DOMAĆINA

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SADRŽAJ

U radu su izneti rezultati proćavanja lokalnog imunološkog odgovora pilića kao nespecifićnih domaćina posle infekcije sa kokcidijom *E. colchici* specifićnom za fazane. Kod pilića ova kokcidija naseljava i kranijalne partije creva (duodenim) a ne samo cekum kao kod fazana. U sluzokoži ovih delova creva dokazano je povećanje broja eozinofilnih granulocita i CD3 + T ćelija počevši od 36 sata nakon infekcije. Ovo govori u prilog hipotezi da su CD3+ limfociti ukljućenog u nastanak imunog odgovora i da mogu imati ulogu u sprećavanju daljeg razvoja *E. colchici* u telu nespecifićnog domaćina.