Changes in intestinal intra-epithelial and systemic T-cell subpopulations after an Eimeria infection in chickens : comparative study between E. acervulina and E. tenella

M. Bessay, Yves Le Vern, Dominique Kerboeuf, P. Yvore, Pascale P. Quéré

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Changes in intestinal intra-epithelial and systemic T-cell subpopulations after an *Eimeria* infection in chickens: comparative study between *E acervulina* and *E tenella*

M Bessay [1], Y Le Vern [2], D Kerbœuf [2], P Yvoré [1], P Quéré [3*]

[1] *Laboratoire de protozoologie;*
[2] *Service commun de cytométrie;*
[3] *Laboratoire de virologie aviaire et d'oncologie, station de pathologie aviaire et parasitologie, Inra, 37380 Nouzilly, France*

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Summary — During chicken coccidiosis, the growth of the parasite in the intestinal epithelium cells leads to the development of host immune response. Cell-mediated immune mechanisms appear to be mainly responsible for the acquired resistance to disease. The action of two species of *Eimeria*, with two different intestinal localizations, on T-lymphocyte subsets was followed by fluorescent antibody cell-sorter analysis, locally at the intestinal site of the parasitic development and systemically in spleen and blood. An *Eimeria acervulina* infection, localized in duodenum, induced a significant increase in the proportion of $CD4^+$ (up to 15%), $CD8^+$ (up to 12%) and TCRγ/δ (up to 6%) in the duodenal intraepithelial leucocytes (IEL) from day 4 to day 8 PI, and an increase in the proportion of IgM^+ cells (12%) on day 8. At the same time, the proportion of $CD8^+$ cells dropped significantly in the blood and spleen (–5 to –10%) on days 4 and 6 PI and then increased with the proportion of $CD4^+$ cells on day 8. An *E tenella* infection, localized in caecum, increased the proportion of $CD4^+$ cells on day 8 PI (20%) and of $CD8^+$ cells (10%) on days 6 and 8 PI in caecal IEL. A negative or zero effect on the proportion of TCRγ/δ+ cells was observed as well as on the IgM^+ cells. At the same time, the proportion of $CD4^+$ cells dropped in the spleen on day 8 PI (–10%) and that of $CD8^+$ cells dropped in the blood on day 6 (–15%). In conclusion, *Eimeria* infection seems to rapidly induce, locally at the site of the parasite development, a dramatic modification of the proportion of T-cell subsets in IEL, accompanied by systemic variations that are generally opposing, in the lymphocyte populations. The timing of the changes seems to follow the phases of the parasitic cycle for the *Eimeria* species considered.

chicken / *Eimeria acervulina* / *Eimeria tenella* / intraepithelial leukocyte / immunity

* Correspondence and reprints

Résumé — Modifications des sous-populations de cellules T intraépithéliales intestinales et systémiques après infection chez le poulet. Comparaison entre *E acervulina* et *E tenella*. *Les coccidies aviaires se développent dans les cellules épithéliales de l'intestin. La multiplication parasitaire conduit à l'apparition d'une réponse immune chez l'hôte, dont les mécanismes d'immunité à médiation cellulaire paraissent être les principaux responsables de la résistance acquise. L'effet de l'infection par deux espèces d'*Eimeria *à localisations intestinales différentes, sur les populations lymphocytaires T, a été suivi par cytométrie de flux, localement au lieu de multiplication parasitaire, dans le sang et dans la rate. L'infection par* E acervulina, *se développant dans le duodénum, induit une augmentation significative du pourcentage des lymphocytes CD4+ (jusqu'à 15 %), CD8+ (jusqu'à 12 %) et TCRγ/δ+ (jusqu'à 6 %) dans les leucocytes intraépithéliaux du duodénum du quatrième au huitième jour suivant l'inoculation, de même qu'une augmentation des cellules IgM+ (12 %) au huitième jour. Parallèlement, le pourcentage de lymphocytes CD8+ chute significativement dans la rate et le sang (–5 à 10 %) du quatrième au sixième jour suivant l'inoculation, puis s'accroît avec le pourcentage de lymphocytes CD4+ au huitième jour. Par comparaison, l'infection par* E tenella *induit une augmentation du pourcentage des lymphocytes CD4+ au huitième jour (20%) et CD8+ du sixième au huitième jour suivant l'inoculation (10%) dans les leucocytes intraépithéliaux du caecum. Un effet négatif ou pas d'effet n'est observé sur le pourcentage des cellules TCRγ/δ+ ou IgM+. Parallèlement, le pourcentage de lymphocytes CD4+ chute dans la rate au huitième jour (–10 %) et celui des lymphocytes CD8+ chute dans le sang au sixième jour suivant l'inoculation (–15 %). En conclusion, l'infection par* Eimeria *modifie rapidement et fortement la proportion des sous-populations T dans les lymphocytes intraépithéliaux du lieu du développement parasitaire, obtenus par une technique d'isolement. Elle s'accompagne d'une modification, en général inverse, de ces mêmes populations au niveau systémique (sang et rate). La chronologie de ces modifications suit les phases du cycle parasitaire (période prépatente).*

poulet* / Eimeria acervulina / Eimeria tenella / *leucocyte intraépithélial* / *immunité

INTRODUCTION

Avian coccidiosis in chickens is caused by intracellular protozoan parasites from seven species of the genus *Eimeria* (*E acervulina, E tenella, E praecox, E maxima, E brunetti, E mitis* and *E necatrix*) that develop in the intestinal epithelium. Host responses to coccidian parasites are complex and involve both humoral and cellular mechanisms. Studies on the protective immunity of chickens to coccidiosis underline the preponderant role of cell-mediated immune responses (Rose and Hesketh, 1982; Lillehoj, 1987; Lillehoj and Trout, 1993). Nevertheless, the paucity of chicken immune reagents available does not permit a clear understanding of the immune reactions and cytokine network such as is possible in mammals. A better understanding of the immune mechanisms and the cells involved in the protection against coccidiosis is necessary as a crucial step toward the development of adapted vaccines, especially those using recombinant proteins developed using genetic engineering.

Sporozoites enter the intestinal mucosa by penetrating villus epithelial cells, but then complete the first generation schizogony in the crypt epithelial cells, with the exception of *E brunetti* and *E praecox*. During their migration through the mucosa, the sporozoites encounter cells from the immune system. Attention has focused in particular on the possible involvement of cells from the immune system such as T-lymphocytes and macrophages in possibly transporting the sporozoites (Lawn and Rose, 1982; Lillehoj and Chung, 1992; Trout and Lillehoj, 1993; Vervelde et al, 1995).

Host immune responses are triggered during the sporozoite migration as the parasite cycle progresses, and a strong protective immunity generally takes place after a primary infection (for a review, see Shirley, 1992). Intraepithelial (IEL) and lamina propria (LPL) leucocytes are the first line of defence in the intestine. Little information

is available as yet on the chicken intestinal immune system. But the phenotypic characterization of cells from the chicken intestinal immune system reveals an organization similar to that in mammals (Bucy et al, 1988; Chai and Lillehoj, 1988; Hoggenmüeller et al, 1993).

The purpose of our study was to follow the changes in T-cell subsets in IEL following a primary infection with Eimeria at the site of parasite multiplication. Moreover, the general impact of infection was also studied on the systemic immune system (blood and spleen) and other areas of the intestine. A comparison was performed between two species that localize in two distinct areas of the intestine: *E acervulina,* which develops in the duodenum, and *E tenella* which develops in the caeca.

MATERIALS AND METHODS

Chickens and parasites

Male White Leghorn chickens of histocompatible line (GB1) were hatched at INRA, Nouzilly, and raised on wire-floored cages under specific pathogen free (SPF) conditions until use. Feed and water were provided ad libitum. At eight weeks of age, they were randomly distributed into treatment groups according to the experimental design. Two species of *Eimeria* were used: *E acervulina* (strain PAPa46) and *E tenella* (strain PAPt36). These two strains were maintained by successive passages in vivo.

Cell preparation

Blood cells

Blood was removed by intracardiac puncture. After an incubation step (45 min, 37 °C), the blood was centrifuged at low speed (5 min, 45 *g*). Plasma was removed and the cells were concentrated after further centrifugation (10 min, 400 *g*). Viable cells were numbered in the presence of trypan blue.

Spleen cells

The spleens were removed and gently teased through a 40 µm-mesh steel screen in 1.1 equiv PBS (phosphate buffer saline, pH 7.4, Gibco). The cells were collected after centrifugation (20 min, 210 *g*), then resuspended in 1.6 mL PBS. Viable cells were numbered in the presence of trypan blue.

Intraepithelial leukocytes

The preparation of intraepithelial leukocytes is based on a modification of a previously described technique (Chai and Lillehoj, 1988). The duodenum (c loop, 12–15 cm) and the caeca were washed in a HBSS medium (Hank's Balanced Salt Solution, Gibco) supplemented with glucose (4 g/L) and foetal calf serum (FCS, 2%, Gibco) (medium HBSS1). The intestinal portions were then cut in HBSS1 medium and incubated for 10 min at room temperature in HBSS1 containing dithiothreitol (DTT, 2 mM, Gibco). This step eliminates intestinal mucus. The supernatant was discarded and the small pieces of intestine were incubated twice for 20 min at 41 °C in HBSS1 containing 2 mM DTT and 3 mM EDTA (ethylenediaminetetraacetic acid, Gibco). The supernatant was centrifuged for 2 min at 45 *g*. The medium containing the leukocytes was passed through a glass-wool column. This step eliminates most epithelial cells and cellular clusters. The cells were then centrifuged on Ficoll ($d = 1.077$, 30 min, 490 *g*) to remove red cells. The intraepithelial leukocytes were washed several times and counted.

Flow cytometric analysis

Spleen or blood cells (2×10^6) and 20 000–800 000 intestinal cells were centrifuged for 3 min at 400 *g*. The supernatant was discarded and 100 µL monoclonal antibody was added to the cells. The monoclonal antibodies used to mark the chicken T-lymphocytes recognize the CD4 or CD8 (Chan et al, 1988) or TCRγ/δ (Bucy et al, 1988) cell surface antigen and were kindly provided by MD Cooper and CL Chen (Departments of Medicine, Pediatrics and Microbiology, University of Alabama at Birmingham, AL). The monoclonal antibody which recognizes class II molecules at the surface of B-lymphocytes, activated T-lymphocytes and macrophages (Guille-

mot et al, 1986) was kindly provided by N Le Douarin (Collège de France and CNRS, Institut d'embryologie, Nogent s/Marne, France). The monoclonal anti-IgM antibody was purchased from Janssen (Noisy-Le-Grand, France). The 9D4 monoclonal antibody, kindly provided by F Coudert (Laboratoire de virologie et d'oncologie aviaires, INRA, Tours-Nouzilly, France), stains by fluorescence 90% thymus cells, 45–50% bursa cells, 90% blood cells (obtained by low speed centrifugation) and 70% spleen cells, and is used as a pan-lymphocyte marker. After an incubation step (30 min, 4 °C) and two washes in PBS with 2% FCS, a goat polyclonal antibody directed against mouse IgG was added. For the blood and spleen cells it was conjugated with fluorescein (used at a dilution of 1:40, Nordic) and for the intestinal leukocytes it was conjugated with phycoerythrin (used at a dilution of 1:20, Caltag). After another incubation step, 100 µL of 1% paraformaldehyde was added to fix the cells. The fluorescence analysis was performed with a FACS Star Plus (Becton Dickinson).

Experimental design

Two groups of chickens were used: a control group (four chickens) and an infected group (eight chickens). The chickens were infected with 10^5 oocysts of *E acervulina* or 2 x 10^4 oocysts of *E tenella*. The blood, spleen, duodenum and caeca were removed 2, 4, 6, 8 and 14 days after infection. For practical reasons, a first experiment was conducted for the analysis of the blood and spleen lymphocytes, and a second one for the analysis of the duodenal and caecal lymphocytes. The results are expressed as:

% = % infected group – % control group

Statistical analysis

The Student's *'t'* test was used to compare the values obtained in the control group with the values obtained in the infected group.

RESULTS

Phenotypic characterization of IEL populations obtained from duodenum or caecum of GB1-histocompatible chickens

In the duodenum, as in the caecum, the percentage of $CD8^+$ cells was higher than the percentage of $CD4^+$ cells. We found a lower percentage of $TCR\gamma/\delta^+$ cells in the duodenum than in the caecum. The percentage of IgM^+ cells in the caecum was higher than

Table I. Percentage of the different cell populations in the duodenal and caecal leucocyte preparations and in blood and spleen from control GB1 chickens.

	9D4[a]	*CD4*	*CD8*	*TRCγ/δ*	*IgM*	*Class II*
Duodenum	65.63 ± 9.24[b]	16.75 ± 4.21	27.20 ± 7.26	9.33 ± 2.66	6.95 ± 4.15	44.02 ± 6.89
Caecum	78.49 ± 7.49	25.61 ± 6.20	36.94 ± 7.8	20.99 ± 4.27	14.60 ± 4.99	42.14 ± 6.29
Blood	ND[c]	47.60 ± 2.41	29.10 ± 1.54	ND	22.22 ± 2.00	13.55 ± 1.57
Spleen	ND	23.27 ± 2.84	47.26 ± 2.04	ND	26.54 ± 0.66	40.02 ± 1.67

[a] The monoclonal antibodies used were: 9D4 as a pan-lymphocyte marker; CD4, CD8 and TCRγ/δ as markers of T-cell subpopulations; IgM as a marker of B-lymphocytes and TAP as a marker of class II+ cells. [b] Mean ± SEM (n = 8). [c] ND = Not done.

the percentage of the same cells in the duodenum. For the class II+ cells, the percentage in the duodenum was about the same as the percentage in the caecum. These results are summarized in table I.

Changes in the total number of IEL obtained from the duodenum or caecum during infection with Eimeria

During the first two days, a slight drop of the number of IEL in the infected chickens compared with the control ones was observed (fig 1). Eight days after infection, the number of cells in the infected group was significantly higher than the number of cells in the control group (+1200 for an infection with *E acervulina* and +4200 for an infection with *E tenella*). On day 14 PI, the number of IEL in the infected chickens was the same as the number of cells in the control chickens (fig 1). In the caecum of chickens infected with *E acervulina* and in the duodenum of chickens infected with *E tenella*, the number of IEL in the infected group was lower than the number in the control group throughout the experiment until day 14 PI where the number of cells in the two groups was the same (data not shown).

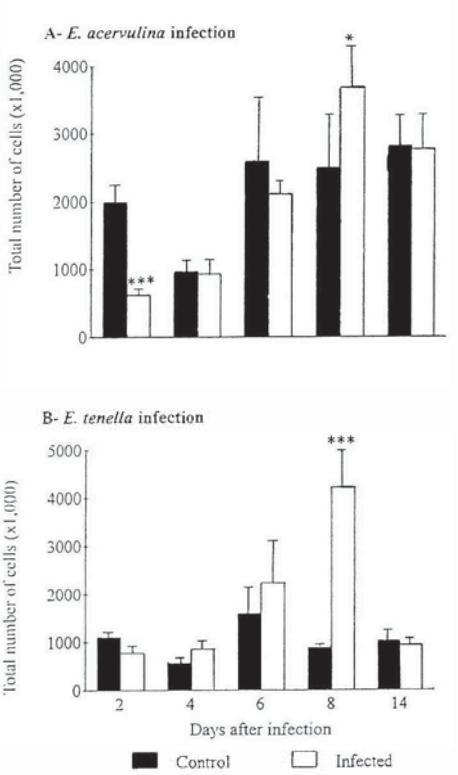

Fig 1. Changes in the number of intraepithelial leukocytes in the duodenum of chickens following an infection with 10^5 *Eimeria acervulina* oocysts (A) or in the caecum of chickens following an infection with 2×10^4 *E tenella* oocysts (B). The number of cells in control chickens and in infected chickens were compared each day using a Student's *'t'* test. The significance between values is indicated by: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Changes in the percentage of CD4+, CD8+, TCRγ/δ+, IgM+ and class II+ IEL in the duodenum or caecum following an Eimeria infection

T-Lymphocyte subpopulations

As early as day 2 PI, the number of CD4+, CD8+ and TCRγ/δ+ IEL increased in the duodenum of infected chickens with *E acervulina* as compared to the control chickens, and this increase was maintained throughout the 14 days (table II). For CD4+ cells the percentage increase was already significant by day 4 PI, and it reached 17% on day 14 PI. The increase in the percentage of CD8+ cells was also significant on day 4 PI, reached 12% on day 8 PI and then decreased on day 14 PI (table II). As for CD8+ cells, the maximum increase in the percentage of TCRγ/δ+ cells was most significant on day 8 PI. In caecal IEL, the percentages of CD4+, CD8+ and TCRγ/δ+ cells decreased in infected chickens compared with the controls (data not shown).

Table II. Changes in the percentage of CD4+, CD8+, TCRγ/δ+, IgM+ and class II+-IEL in the duodenum of chickens infected with 10^5 oocysts of *E acervulina* and in the coecum of chickens infected with 2 x 10^4 oocysts of *E tenella*.

Days after infection	% *Positive cells* [a]				
	CD4+	*CD8+*	*TCRγ/δ+*	*IgM+*	*Class II+*
			E acerverlina		
Duodenum					
2	6.27	1.92	0.84	−3.15	−19.85*
4	14.19**[b]	6.14*	0.45	1.51	−11.77***
6	5.92	2.61	4.57*	6.95*	−12.28**
8	13.14***	12.14**	6.24*	12.21**	1.21
14	17.36**	8	4.28	3.95	−11.21*
			E tenella		
Coecum					
2	5.28	10.53	−1.61	6.82	−3.81
4	−0.38	−1.85	−5.97	−4.57	−6.41
6	−1.3	7.17*	−8.22**	−9.48**	−20.19**
8	26.19**	4.49	−11.88	4.64	7.44
14	4.66	−2.33	−4.41	−2.41	11.53

[a] Results are expressed as the difference between the mean percentage of positive cells for the infected group (six to eight chickens) and the control group (four chickens). SEM represents 10–25% of the mean for the infected group and the control group depending on the day. [b] Statistical significance using a Students *'t'* test of the difference between the infected group and the control group is indicated by: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

In caecal IEL preparations of chickens infected with *E tenella*, a peak was observed relating to the CD4+ cell percentage at day 8 PI (26%). For CD8+ cells, an increase of 10% was noted as early as day 2 PI, followed by another increase on day 6 PI (7%) and day 8 (5%). No further effects were seen on day 14 PI. For TCRγ/δ+ cells, a decrease was observed in the percentage level that became significant on day 6 PI. In comparison, the percentage of CD4+ and CD8+ cells were lower in the duodenal IEL preparations for infected chickens compared with control ones (data not shown).

IgM+ cells

In the duodenal IEL preparations from chickens infected with *E acervulina*, a significant increase in the number of IgM+ cells was observed on day 6 PI, reaching 12% on day 8 PI. No effect was seen on the number of IgM+ cells in the caecal IEL preparations throughout the experiment (data not shown).

The percentage of IgM+ cells in caecal IEL preparations from chickens infected with *E tenella*, decreased significantly on day 6 PI, and returned to almost normal values thereafter. For the duodenal IEL preparations, the values for the two groups of chickens were the same (data not shown).

Class II+ cells

In the *E acervulina* infection, the percentage of class II+ cells in the duodenal IEL preparations from infected chickens was lower than from control chickens from day 2 to

day 6 PI, returned to normal levels on day 8 PI and decreased again on day 14 PI. No effect was seen on the caecal IEL preparations (data not shown).

After *E tenella* infection (table II), the percentage of class II$^+$ cells was also lower in caecal IEL preparations from infected chickens from day 2 to day 6 PI and increased on days 8 and 14. No effect was seen on the duodenal IEL preparations (data not shown).

Effect of Eimeria *infection on the percentage of CD4$^+$, CD8$^+$, IgM$^+$ and class II$^+$ cells in blood and spleen*

In the blood of chickens infected with *E acervulina* a peak in the level of CD4$^+$ lymphocytes was observed on day 8 PI compared to control (table III). For the other days, the difference between the percentage of cells in infected chickens and in control chickens was not significant. For the levels of CD8$^+$ lymphocytes and IgM$^+$ cells, the differences between infected and control chickens were not significant except on day 4 PI for the former. For the class II$^+$ cells, an increase was observed on day 6 PI. No significant effect was observed in the spleen, whatever the monoclonal antibody used.

In the blood of chickens infected with *E tenella*, there were no significant differences between infected and control chickens for CD4$^+$, IgM$^+$ or class II$^+$ cell levels. For CD8$^+$ lymphocytes, the minimal values were obtained on days 6 and 8 PI. On day 14 PI, the percentage of CD8$^+$ cells was higher in infected chickens than in control chickens. In the spleen, an increase in CD4$^+$ cells was obtained on day 6 PI, followed by a decrease on day 8 PI. For CD8$^+$ cells, the percentage in the infected group was lower than in the control group throughout the experiment. No great variation in IgM$^+$ cell percentage was noted between the two groups of chickens. On day 4 PI there was a decrease in the percentage of class II$^+$ cells in the infected chickens compared to the control chickens. However, on day 8 PI, the percentage of these cells was higher for the infected chickens than for the controls.

DISCUSSION

Because avian coccidia develop in the epithelial cells from the intestinal mucosa, the first line of host defence is constituted by the leukocytes from this area, either located within the epithelium or in the lamina propria. As in mammals, the majority of chicken IEL are T-lymphocytes (Arnaud-Battandier et al, 1980; Bucy et al, 1988; Vervelde and Jeurissen, 1993). They are therefore capable of initiating a cell-mediated immune response following coccidia infection. Studies on the migration of sporozoites in naive chickens have shown that they are first detected in the intestinal villus epithelium after the infection, then in the lamina propria, and subsequently accumulate in the crypt epithelium where they multiply (Vervelde et al, 1995). Leukocytes have been suggested to have a role in the transport of sporozoites to the crypt. Sporozoites have been identified inside such cells as macrophages (Van Doorninck and Becker, 1957; Trout and Lillehoj, 1993) or T-lymphocytes (Vervelde et al, 1995), mainly of the CD8 phenotype (Trout and Lillehoj, 1993, 1995).

In our study, we chose to test the effect of an oral *Eimeria* infection on the intestinal immune system, by considering isolated IEL obtained after the dissociation of the intestinal epithelium (Chai and Lillehoj, 1988; Schwager and Weber, 1992; Hoggenmüeller et al, 1993). A final aim would be to analyse their local role in immunological function studies.

Our results showed that a primary high-dose infection with Eimeria (100 000 oocysts for *E acervulina* and 20 000 oocysts for *E tenella*) in two-month-old histocompatible

GB1 (B13/B13) chickens, led to a drastic modification of their lymphocyte levels, especially the T-cell subsets in the local intestinal area of the parasite development. First of all, the total leukocyte levels of the colonized area of the intestine were affected by the Eimeria infection. After a slight decrease during the first two days of infection, these levels increased steadily until day 8 PI and then returned to normal by day 14 PI. This is quite different from the response observed in mice infected with *E vermiformis,* where a long-lasting depletion of IEL occurred (Findly et al, 1993).

We observed that a significant increase, up to 15% compared with control chickens, occurred in the number of CD4+ cells in duodenal IEL as early as four days after an oral inoculation of *E acervulina,* and was maintained throughout the 14 days PI. The *E*

Table III. Changes in the percentage of CD4+, CD8+, IgM+ and class II+-cells in the blood and in the spleen of chickens infected with 10^5 oocysts of *E acervulina* or 2 x 10^4 oocysts of *E tenella*.

Days after infection	*% Positive cells* [a]			
	CD4+	*CD8+*	*IgM+*	*Class II+*
		E acervulina		
Blood				
2	−3.85	−10.99	−1.84	3.36
4	0.66	−7.96*	−5.89	0.71
6	8.49	−9.29	−0.13	9.11*
8	22.87*[b]	5.02	0.65	6.36
14	0.55	−1.26	0.44	0.78
Spleen				
2	2.99	4.82	0.53	2.31
4	2.05	−0.68	−0.8	3.5
6	−2.02	−6.79	6.59	1.36
8	−5.6	3.82	−0.3	−3.71
14	8.48	−6.3	−4.35	−3.04
		E tenella		
Blood				
2	2.92	−0.89	−3.45	0.43
4	−5.6	−8.15	−6.46	−0.97
6	2.37	−15.19***	−8.64	−1.96
8	3.16	−9.08**	4.79	1.93
14	6.32	5.61*	4.56	−5.39
Spleen				
2	1.45	−2.59	−4.04*	−2.26
4	0.58	−5.96**	−2.73	−12.34**
6	10.21**	−11.94**	5.72*	12.24*
8	−12.65*	−16.52***	4.9	2.64
14	2.23	−8.46	−4.03	2.31

[a] Results are expressed as the difference between the mean percentage of positive cells for the infected group (six to eight chickens) and the control group (four chickens). SEM represents 10–25% of the mean for the infected group and for the control group depending on the day. [b] Statistical significance using a Students '*t* test of the difference between the infected group and the control group is indicated by: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

acervulina infection induced a smaller increase in the number of CD8+ cells, also beginning on day 4 PI and culminating on day 8 PI (up to 12%), and followed by a decrease as early as 14 days PI. The pattern of variation for TCRγ/δ+ cells in this case followed the pattern of CD8+ cells, with a significant increase in numbers reaching 6% on day 8 PI, and a return to normal values thereafter. When compared with the few studies present in literature, the percentage of CD8+ cells we obtained for control chickens by isolating IEL (27%), was less than that of Lillehoj and Bacon (1991) and Lillehoj (1994), 37–53%, but the percentage of CD4+ cells was a little higher, 17% compared to 10%. Nevertheless, the number of TCRγ/δ+ cells we found in the control duodenum, about 10% of the total IEL, was far lower than values in the literature, 33–45% (Bucy et al, 1988; Lillehoj and Chung, 1992). Literature on the subject has underlined the great variability in the percentage of CD4+, CD8+ and TCRγ/δ+ cells in intestines. These differences are due to the ages of the chickens or genetic differences (Bucy et al, 1988; Lillehoj and Chung, 1992; Lillehoj, 1994). To sum up, compared with the study of Lillehoj (1994), after a primary *E acervulina* infection, the increase we observed in the GB1 line in duodenal IEL for CD4+ cells followed the amplitude and the pattern of the TK line, which is very sensitive to coccidiosis, but the one for CD8+ cells and TCRγ/δ+ cells followed the amplitude and the pattern of the SC line, which is more resistant to coccidiosis and in any case has a maximum on day 7 PI. So, for the present, it remains difficult to connect a phenotype of resistance to the triggering of specific intestinal T-cell subsets for *E acervulina*.

The primary oral infection with *E tenella*, led to somewhat different results regarding the variation in the percentage of CD4+, CD8+ and TCRγ/δ+ cells in the caecal IEL. The increase in CD8+ cells appeared earlier, at day 2 PI, then another peak (6–10%) was observed from days 6 to 8 PI. No increase in the level of TCRγ/δ+ cells was noted throughout the 14 days of the experiment. A large increase in the number of CD4+ cells (up to 25%) occurred on day 8 PI, followed by a severe decrease on day 14 PI. The percentages of CD4+ cells (26%) and CD8+ cells (37%) were slightly higher than those found in the literature for control chickens (Lillehoj and Chung, 1992 ; Lillehoj, 1994). However the percentage of TCRγ/δ+ cells (21%) was lower. Our results are in agreement with those available in the literature for *E tenella* infection using the same isolation technique (Schwager and Weber, 1992). These authors observed an increase in the number of CD4+ cells on days 5 to 6 PI, but no change for CD8+ cells.

For other cells such as IgM+ cells, we observed a rather low number in the duodenal IEL (7%). This was a little higher in the caecum (15%). Immunohistological studies on chicken intestine underline the scarcity of B-cells in epithelium (Jeurissen et al, 1989). The presence of some IgM+ cells in our preparation could be a sign of possible contamination with cells from the lamina propria. Hoggenmüeller et al (1993) found 4–5% plasma cells in isolated IEL from the small intestine using the same technique. Others did not determine the number of IgM+ cells in their duodenal IEL preparations (Lillehoj, 1994). Nevertheless the CD4+/CD8+ cell ratio in our duodenal IEL is in agreement with the literature (Bucy et al, 1988). No datum is available on isolated caecal IEL. After an *E acervulina* infection, the number of IgM+ cells increased significantly by days 6 to 8 PI in the duodenal IEL preparations. But no such increase was noted after an oral *E tenella* infection in the caecal IEL up to 14 days PI. Moreover, no increase in the number of class II+ cells was noted after an oral *E acervulina* infection in duodenal IEL throughout the 14 days PI, but an increase was observed, in caecal IEL number after an oral *E tenella* infection, as seen by Schwager and Weber (1992).

Using a different technique, such as immunohistochemical analysis studying the variation of T-cell subsets 'in situ' at the local site of parasite multiplication, gave rather different results. Trout and Lillehoj (1995) found that an *E acervulina* infection induced an increase in $CD8^+$ cells in the duodenal lamina propria as soon as 24 h PI but had little effect in the epithelium, and then very few changes occurred up to 72 h PI. Few $CD4^+$ cells were observed in the lamina propria. On the other hand, Rothwell et al (1995) showed that an oral *E maxima* in the jejunum induced a very high accumulation of $CD4^+$ cells and $TCR\alpha/\beta^+$ cells specifically in the lamina propria concomitantly with an increase of $CD8^+$ cells and $TCR\gamma/\delta^+$ cells in the epithelium 10 days PI (ie, at the end of the prepatency for this coccidia). In their work the IgM^+ cells exhibited an early increase in the days just following infection, and then another ten days later.

It is rather difficult at present to draw a general picture of which modifications of the immune system appeared in the intestine, especially at the local area of parasite multiplication, as a result of a primary infection with avian coccidia. The literature points to discrepancies among authors, first of all due to the different techniques used (isolation procedures to obtain leukocytes from the intestinal epithelium or immunohistochemical studies, but also due to the kind of chickens used, their genetic origin and age, and the doses of coccidia used (high or low). Immune modifications taking place after a primary infection are quite different from those following a second inoculation (Trout and Lillehoj, 1995; Vervelde et al, 1995; Rothwell et al, 1995). As a first line of defence against coccidiosis, NK cells (Chai and Lillehoj, 1988; Lillehoj, 1989) and $TCR\gamma/\delta^+$ cells (Trout and Lillehoj, 1995; Rothwell et al, 1995) have been considered to be important immediately after the parasitic invasion, as for the case of some intestinal parasites in mammals. For *E acervulina* in isolated duodenal IEL, we found a parallel increase in $CD8^+$ cells and $TCR\gamma/\delta^+$ cells from the middle to the end of the prepatent period, similar to what was observed in jejunum epithelium for *E maxima* (Rothwell et al, 1995). No effect on $TCR\gamma/\delta^+$ cells was noted for *E tenella* in coecal IEL, despite a slight increase of $CD8^+$ cells. This might reflect a possible difference in the early immune response involved according to the parasitized area, ie, the upper or lower part of the intestinal tract, but this hypothesis needs confirmation. Moreover our results place particular emphasis on $CD4^+$ cells; during infection these seemed to be strongly triggered at the local areas of parasite multiplication, the maximum levels of these cells being observed in IEL around the end of the prepatent period (4.5–5 days for *E acervulina* and 6–8 days for *E tenella*). This corroborates the observation of Rothwell et al (1995) with *E maxima*, but in lamina propria. The end of the prepatent period coincides with oocystal excretion and the establishment of immunity, greatly limiting further parasite multiplication.

Due to the lack of data in the literature, we were particularly interested to follow the variations caused by the primary infection with *Eimeria* in chickens, in the T-cell subsets in other compartments of the immune system, such as an intestinal area different from that colonized by the parasite, or the systemic compartments (blood and spleen). In every case, the infection with *Eimeria* induced a significant decrease in all T cell-subsets in the caecum for *E acervulina* and in the duodenum for *E tenella* (data not shown) corresponding to the prepatent period. At the same time, the number of systemic $CD8^+$ cells was particularly depressed during the four days preceding the end of the prepatent period for each *Eimeria*. This was seen in blood for *E acervulina*, when infected chickens increased their duodenal $CD8^+$ IEL, but even more in blood and spleen for *E tenella* despite the lower effect

of infection on coecal CD8+ IEL. Considering CD4+ cells, their number was first depressed and then increased around the prepatent period (significantly for *E acervulina* at day 8 PI). The same results were obtained by Schwager and Weber (1992) with *E tenella*. It is noteworthy that the appearance of a systemic cell-mediated immune response, revealed by antigen-specific proliferation studies (Lillehoj, 1986; Martin et al, 1993, 1994), takes place as soon as 7 days PI for *E acervulina* and *E tenella*. Moreover the local antibody immune response is significant as soon as 7 days PI for *E tenella* (Zigterman et al, 1993) and *E acervulina* (personal observation) in the intestinal segments colonized by the parasite, but not in the other intestinal areas. This demonstrates an already high and effective stimulation of the immune system, involving the stimulation of CD4+ cells for antigen-specific lymphocyte proliferation and the antibody production. In fact, studies in mice underline the essential role of CD4+ cells in controlling a primary *E vermiformis* infection (Rose et al, 1988, 1992). Compared with several histocompatible chicken lines, the GB1 line interestingly exhibited the highest specific antibody response in serum following an *E tenella* infection, concomitant with the lowest oocyst production, despite showing the highest sensitivity to the disease (M Naciri, personal communication).

In conclusion we observed, with two different *Eimeria* located at distinct intestinal areas, duodenum and coeca, the same general pattern of lymphocyte changes following a primary infection of GB1 chickens. At the local area of the parasitic multiplication, T-lymphocytes seemed to be triggered quickly during the middle to end of the prepatent period as was shown by the increase in the total number of leukocytes, a little earlier for CD8+ cells and then especially for CD4+ cells. In other intestinal areas, this positive effect was concomitant with (or somewhat preceded by in the systemic compartment), a negative effect seen on the CD4+ and especially CD8+ T-cell subsets. So the local changes in lymphocyte subsets in the intestinal area where the Eimeria multiplication takes place are probably due to a powerful local triggering of lymphocyte stimulation and proliferation, but the immediate participation of cells from other intestinal areas, and also from the systemic compartment, seems to be significant in the case of a high level of infection. Further studies of the lymphocyte migration pattern are necessary to clarify this point.

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