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Profiling local gene expression changes associated with *Eimeria maxima* and *Eimeria acervulina* using cDNA microarray

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Abstract *Eimeria* parasites show preferential sites of invasion in the avian intestine and produce a species-specific host immune response. Two economically important species, *Eimeria acervulina* and *Eimeria maxima*, preferentially invade and develop in the avian duodenum and jejunum/ileum, respectively. To investigate local host immune responses induced by parasite infection, global transcriptional changes in intestinal intraepithelial lymphocytes (IELs) induced by oral inoculation of chickens with *E. acervulina* or *E. maxima* were monitored using cDNA microarrays containing 400 unique chicken genes. Multiple gene transcripts were significantly up- or down-regulated following primary or secondary infection with *E. acervulina* or *E. maxima*. In general, infection by either parasite resulted in the expression changes of more genes following primary infection than following secondary infection, and *E. acervulina* caused more changes than did *E. maxima*. Although different regions of the small intestine were infected, similar changes in the levels of several cytokine mRNAs were observed in both *Eimeria* species following primary infection. Also identified was a set of transcripts whose expression was commonly enhanced or repressed in intestinal IELs of chickens infected with either parasite. Microarray analysis of chicken genes induced or repressed following *Eimeria* infection offers a powerful tool to enhance our understanding of host–parasite interactions leading to protective immunity.

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Introduction

Avian coccidiosis is caused by an intestinal protozoa belonging to several species of the genus *Eimeria*. Protozoan infection results in extensive destruction of the avian intestinal epithelium accompanied by reduced feed efficiency, severe depression in body weight gain, and thus significant economic losses for the poultry industry (Lillehoj 1998; Lillehoj and Lillehoj 2000). Although avian coccidiosis has been extensively controlled by prophylactic medication, alternative control methods are needed due to the emergence of drug-resistant parasite strains (Chapman 1993) and the uncertain future for drug usage in animal husbandry. In this regard, considerable efforts have been made to develop recombinant vaccines (Barriga 1994; Lillehoj et al. 2000; Song et al. 2000; Min et al. 2001). However, control of coccidiosis by field vaccination has been only partially successful due to limited information on host protective immunity, antigenic variation associated with different *Eimeria* life-cycle stages, and the appearance of mutant strains (Fitz-Coy 1992; Tomley 1994). Recent studies investigating chicken adaptive immune effector mechanisms suggest that field vaccination to control of *Eimeria* infection may be commercially feasible. This prospect is exemplified by the studies of the intestinal immune response produced subsequent to *Eimeria* infection (Jakowlew et al. 1997; Choi et al. 1999; Yun et al. 2000a, b; Laurent et al. 2001; Li et al. 2002; Miyamoto et al. 2002). It is now known, for example, that intestinal intraepithelial lymphocytes (IELs) clearly play a central role in protective immunity to avian coccidiosis (Lillehoj and Lillehoj 2000).

Recent advancements in high-throughput gene profiling technology have made it possible to monitor the expression of thousands of gene transcripts in a single hybridization experiment (Freeman et al. 2000) thereby

making a significant contribution to an understanding of the molecular processes associated with health and disease (Khan et al. 1999; Young 2000; Lucchini et al. 2001). DNA microarrays have been successfully applied to monitor changes in gene expression following infection with viruses (Morgan et al. 2001), bacteria (Cohen et al. 2000), and parasites (Blader et al. 2001; de Avalos et al. 2002). In light of the recent availability of chicken-tissue-expressed sequence tag (EST) libraries from various tissues, we applied high-throughput gene expression profiling to systematically study host–pathogen interactions in avian coccidiosis, with particular focus on chicken genes associated with protective immunity. In this report, our initial results using cDNA microarray analysis of avian intestinal gene expression changes following primary and secondary infection of chickens with *E. acervulina* or *E. maxima* are described.

Materials and methods

Animal, parasites, and experimental infection

Fertilized eggs of White Leghorn SC chickens were purchased from HyVac (Adel, Iowa, USA) and hatched at the Animal and Natural Resources Institute (Beltsville, Md., USA). Hatched chicks were given free access to feed and water and constant light was provided during the entire experimental period. Wild-type strains of *E. acervulina* and *E. maxima*, developed and maintained in the Parasite Biology, Epidemiology and Systematics Laboratory (Beltsville, Md., USA), were cleaned by flotation on 5.25% sodium hypochloride and washed three times with Hanks' balanced salt solution (HBSS, Sigma, St. Louis, Mo., USA). Chickens in the experimental group (n=192) were orally infected at 3 weeks of age with 1×10^4 sporulated oocysts of *E. acervulina* or *E. maxima*. Secondary infection was given orally at 9 weeks of age with 2×10^4 sporulated oocysts of the homologous parasite. Chickens in the experimental group were evenly divided into four groups (n=48) for primary or secondary infection with *E. acervulina* or *E. maxima*. Twelve chickens orally inoculated with HBSS alone served as negative controls.

Preparation of RNA from intestinal IELs

Since *E. maxima* infection results in more severe intestinal pathology than infection with *E. acervulina*, and oocyst shedding begins at day 5 post-infection (Conway and McKenzie 1991; Choi et al. 1999), IELs were collected from the duodenal region (for *E. acervulina*) or the section from Meckel's diverticulum to the ileac region (for *E. maxima*) at 1, 2, 3, and 4 days (12 chickens/day) following primary and secondary infections as described previously (Min et al. 2001). Total RNA from IELs was prepared using TRIzol (Life Technologies, Gaithersburg, Md., USA) and poly(A)$^+$ RNA isolated using the PolyA Tract mRNA Isolation System IV (Promega, Madison, Wis., USA).

cDNA microarrays

cDNA clones (n=2,600) selected from the chicken EST database (http://www.chickest.udel.edu) were applied to nylon membrane using the procedure of Morgan et al. (2001). A subset of these (n=388) was selected as described below, transferred to 100 μl of Luria-Bertani medium containing 100 μg ampicillin/ml in 96-well plates, and incubated overnight at 37 °C. Approximately 1 μl of each culture was removed for PCR amplification using vector primers, and the products analyzed on 1% agarose gels. PCR products were precipitated in ethanol, washed, dissolved in 50% dimethyl sulfoxide, and spotted onto CMT-GAPS-coated glass slides (Corning, Corning, N.Y., USA) using a Cartesian robot model PlySys 5500 PA workstation (Cartesian Technologies, Irvine, Calif., USA) equipped with a Telechem printhead and stealth quill pins.

cDNA probes, hybridization, and data analysis

To prepare hybridization probes, 10 μg of total RNA was mixed with 2.0 μg of oligo (dT), heated for 10 min at 70 °C, and place on ice for 2 min. A cocktail containing 0.5 mM each of dATP, dGTP, and dTTP, 0.1 mM dCTP, 0.1 mM Cy3-dCTP or Cy5-dCTP (Amersham Pharmacia Biotech), 0.5 mM dithiothreitol, and 400 units of Superscript II (Life Technologies) was added. The mixture (30 μl) was incubated at 42 °C for 2 h; labeled probes were purified on Probequant G-50 microcolumns (Amersham Pharmacia Biotech), precipitated, and resuspended in 10 μl of TE buffer (10 mM Tris-HCl, 1.0 mM ethylenediamine tetraacetic acid, pH 8.0). For hybridization, 10 μl each of the Cy3- and Cy5-labeled probes were mixed with 24 μl of hybridization buffer [25% formamide, 5× saline–sodium citrate (SSC), 0.1% SDS, 20 μg human Cot-1 DNA, and 20 μg poly(dA)$_{10}$] (DeRisi et al. 1996), the mixture heated for 5 min at 95 °C, and cooled to 42 °C.

Microarray slides were fixed under UV irradiation, incubated in prehybridization buffer (25% formamide, 5× SSC, 0.1% SDS, 1% bovine serum albumin) for 45 min at 42 °C, rinsed with distilled water, and dried. Slides were hybridized with labeled probes for 18 h at 42 °C, then washed twice with 2×SSC containing 0.1% SDS for 4 min at 42 °C, and three times with 0.1×SSC for 2 min at room temperature. After a brief centrifugation at 500 g, fluorescence intensities of the slides were measured using a ScanArray 4000 scanner (Packard BioScience, Billerica, Mass., USA) and the data normalized by within print-tip group normalization (http://www.stat.berkeley.edu/users/terry/zarray/Html/normspie.html). Statistical significance was determined using the Significance Analysis of Microarrays (SAM) software (http://www.stat.stanford.edu/~tibs/SAM/index.html) as described (Tusher et al. 2001).

Results

Microarray construction

Poly(A)$^+$ RNA isolated from chicken intestinal IELs at 4 days following primary and secondary infection with *E. maxima* was labeled with ^{32}P and hybridized to cDNA nylon microarrays containing 1,100 expressed sequence tag (EST) clones from a concanavalin A-activated splenic lymphocyte cDNA library (Tirunagaru et al. 2000) and 1,500 EST clones from a lipopolysaccharide (LPS)-activated macrophage (HD11) cDNA library (http://www.chickest.udel.edu). From the means of two independent hybridization studies, 388 clones (292 concanavalin A-induced, 96 LPS-induced) were selected that showed at least 1.5-fold increased or decreased transcript levels compared with uninfected controls after global normalization (http://www.geneindex.org). These 388 clones and 12 chicken cytokine genes previously cloned in our laboratory (Lillehoj and Lillehoj 2000; Min et al. 2001) were PCR amplified and applied in quadruplicate to glass microarrays for further analysis.

Table 1 Number of differentially expressed genes in *Eimeria*-infected chickens

	Primary infection[a]				Secondary infection			
	1[b]	2	3	4	1	2	3	4
Number of induced genes								
Eimeria acervulina	145	167	153	167	110	132	123	150
Eimeria maxima	101	101	91	155	65	114	112	100
E. acervulina + *E. maxima*[c]	77	91	77	124	39	71	59	67
Number of repressed genes								
E. acervulina	142	139	142	158	99	116	99	148
E. maxima	109	105	114	132	73	97	98	112
E. acervulina + *E. maxima*	80	90	87	102	46	44	33	64

[a] Chickens at 3 weeks of age were primarily infected with 1×10^4 sporulated oocyts of *E. acervulina* or *E. maxima* and at 9 weeks of age secondarily infected with 2×10^4 sporulated oocysts of *E. acervulina* or *E. maxima*
[b] Days post-primary and post-secondary infection
[c] Number of identical genes in *E. acervulina* and *E. maxima*

Profiling local gene-expression changes in *Eimeria* infected chickens

To analyze changes in gene expression in the intestine of chickens infected with *E. acervulina* or *E. maxima,* IELs were collected from the duodenum or ileum at 1, 2, 3, and 4 days following primary or secondary infection. Levels of gene transcripts isolated from the IELs were quantified by microarray using print-tip group normalization and analyzed statistically using SAM software (Tusher et al. 2001). Compared with *E. maxima* infection, *E. acervulina* primary and secondary infection resulted in up- or down-regulation of more transcripts, and and either parasite induced changes in a greater number of transcripts after primary infection than following secondary infection (Table 1). Specifically, *E. acervulina* and *E. maxima* infection affected the levels of 99 and 51 gene transcripts, respectively, following primary infection, and 46 and 25 transcripts, respectively, following secondary infection (Fig. 1A). Conversely, *E. acervulina* and *E. maxima* decreased the levels of 88 and 56 gene transcripts, respectively, following primary infection, and 22 and 37 transcripts following secondary infection (Fig. 1B). Comparing primary with secondary infection, *E. acervulina* and *E. maxima* commonly induced 34 and nine transcripts, respectively, and commonly repressed 17 and 24 transcripts, respectively (Fig. 1B).

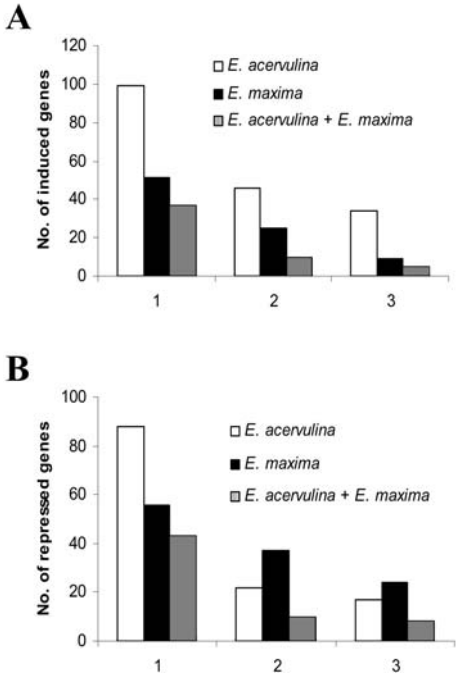

Fig. 1 A list of significantly induced (**A**) and repressed (**B**) genes following *Eimeria* infection. Chickens at 3 weeks of age were infected with 1×10^4 sporulated oocysts of *Eimeria acervulina* or *Eimeria maxima*, and a second infection was given with 2×10^4 sporulated oocysts of the same parasite at 9 weeks of age. At 1, 2, 3, and 4 days following primary and secondary infection, RNA was prepared from intestinal intraepithelial lymphocytes (IELs) obtained from the chicken duodenum (*E. acervulina*) or ileum (*E. maxima*) and used in microarray hybridization. *Bars* Number of identical gene transcripts expressed during all time points of primary (*lane 1*) and secondary (*lane 2*) infection. *Lane 3* Number of identical transcripts expressed at all time points following primary and secondary infection. *Open and closed bars* Number of identical transcripts expressed in *E. acervulina* and *E. maxima*, respectively. *Hatched bars* Number of identical transcripts expressed in both *E. acervulina* and *E. maxima*. The data were obtained from averages of quadruplicate hybridizations and analyzed using SAM software

E. acervulina and *E. maxima* infection induce or repress common gene transcripts

When considering any of the time points examined following primary infection with *E. acervulina* or *E. maxima*, the levels of 37 common transcripts were increased while ten transcripts were induced in common following secondary infection (Fig. 1; Table 2). Conversely, 43 common transcripts were repressed following primary infection and ten were reduced after secondary infection with either parasite (Fig. 1; Table 3). When considering all time points examined following primary or secondary infection with *E. acervulina* or *E. maxima*,

Table 2 Identical genes induced in both *E. acervulina* and *E. maxima* infections. Clone ID comes from http://www.chick-est.udel.edu

Clone ID	GenBank accession	Comments
Identical genes induced in both primary and secondary infections		
pti1c.pk001.a15	AW239651	CMRF35 leukocyte immunoglobulin-like receptor
pat.pk0068.f7.f	AI981915	Zinc finger, C_3HC_4 type
pat.pk0042.c7.f		PmbA homolog
pat.pk0070.c10.f		Granulysin precursor
pmp1c.pk002.h10		Cyclophilin A
Identical genes induced in primary infection		
pti1c.pk001.f3	AW239707	Vitronectin receptor α-subunit precursor
pti1c.pk001.l24		Alcohol dehydrogenase isozyme I
pat.pk0008.h6	AI979992	Enhancer-of-split and hairy-related protein 2
pat.pk0010.g12	AI982328	Elongation factor 1-α
pat.pk0017.c2		Destrine
pat.pk0027.d5.f		Elongation factor 1-α1
pat.pk0027.g9.f	AI980603	Leukotriene A-4 hydrolase
pat.pk0028.c10.f	AI980619	T-cell- receptor γ-V3.8
pat.pk0031.f1.f	AI980712	RANTES protein
pat.pk0032.c10.f	AI980739	Fibronectin
pat.pk0032.h5.f	AI980768	Serine/threonine protein phosphatase PPI
pat.pk0039.b9.f	AI980988	Cytotoxic T-lymphocyte proteinase
pat.pk0042.g8.f		Motopsin
pat.pk0043.c9.f	AI981164	Caspase-3
pat.pk0043.e1.f	AI981169	CD45 homolog
pat.pk0048.d11.f		Guanine nucleotide binding protein (G protein)
pat.pk0048.e5.f	AI981216	CD98 light chain
pat.pk0056.g7.f	AW061440	Monocyte ihibitory receptor
pat.pk0056.c1.f		Cysteine-rich protein
pat.pk0058.a6.f		H3 histone
pat.pk0061.g5.f	AI981638	Centrosomin B
pat.pk0056.a9.f	AI981815	CD36
pat.pk0067.e1.f	AI981862	Acetylcoenzyme A transporter
pat.pk0067.f11.f	AI981870	Mitochondrial thioredoxin-dependent peroxide reductase
pat.pk0068.e7.f	AI981907	MASL1
pat.pk0068.e8.f	AI981908	O-GlcNAc transferase
pat.pk0069.b9.f	AI981932	CD18
pmp1c.pk002.g4		High-affinity IgE receptor γ chain
pmp1c.pk003.d6		Chemokine K203
pmp1c.pk006.a16		Ferritin heavy chain
pmp1c.pk006.i4		Apoptosis-related RNA binding protein
pmp1c.pk006.o10		Nitric oxide synthase
Identical genes induced in secondary infection		
pat.pk0012.c11	AI980066	GRB2 adaptor protein
pat.pk0027.e7.f	AI980592	Protein-tyrosine phosphatase p19
pat.pk0063.c9.f	AI981707	Prolyl 4-hydroxylase β-subunit
pmp1c.pk003.a7		Nitric oxide synthetase 2
pat.pk0023.g8.f	AI980458	Interleukin-8

the quantities of five gene transcripts were commonly induced (CMRF35 leukocyte immunoglobulin-like receptor, zinc-finger gene, PmbA homolog, granulysin precursor, cyclophilin A) and eight were repressed (α-actinin, hypothetical protein F39H12.5, spleen mitotic checkpoint BUB3, interferon-induced granylate-binding protein 2, transcription factor NF-YC subunit, transport associated protein 3, α-adaptin, homobox protein HOX-D8) (Fig. 1; Table 2; Table 3).

Changes in cytokine genes following *Eimeria* infection

Cell-mediated immunity, mediated in part by cytokine production, has been shown to be involved in protection against coccidiosis (Choi et al. 1999; Yun et al. 2000b, c; Lillehoj et al. 2000; Min et al. 2001; Li et al. 2002; Miyamoto et al. 2002). Thus, 12 cytokine genes were included in our microarray analysis in order to monitor changes in their corresponding transcripts subsequent to *Eimeria* infection. As shown in Fig. 2, transcript levels for the interleukin (IL)-8, IL-15, and lymphotactin genes were increased at all time points examined following primary or secondary infection with *E. acervulina* or *E. maxima* whereas IL-18 and osteopontin gene transcripts were repressed. With the exception of transforming growth factor (TGF)-β4, changes in the levels of all cytokine transcripts examined were similar when comparing primary infection by *E. acervulina* with that by *E. maxima*. Similarly, after secondary infection, all transcript levels except those for IL-6 and TGF-β4 showed

Table 3 Identical genes repressed in both *E. acervulina* and *E. maxima* infections. Clone ID comes from http://www.chickest.udel.edu

Clone ID	GenBank accession	Comments
Identical genes repressed in both primary and secondary infections		
pat.pk0002.a2	AI979744	α-Actinin
pat.pk0005.f9	AI979875	Hypothetical protein F39H12.5
pat.pk0008.b12	AI979947	Spleen mitotic checkpoint BUB 3
pat.pk0021.f9	AI980364	Interferon-induced guanylate-binding protein 2
pat.pk0023.f8.f	AI980453	Transcription factor NF-Yc subunit
pat.pk0024.b9.f	AI980475	Transport associated protein 3
pat.pk0041.d12.f	AI981086	α-Adaptin
Identical genes repressed in primary infection		
pco1c.pk001.o23		Single Ig interleukin-1R-related protein
pnf-b.pk0006.a10	AW355358	Macrophage migration inhibitory factor
pft1c.pk002.f6	AW355583	Monocyte to macrophage differentiation protein
pat.pk0002.b12	AI979750	Platelet activating receptor homolog
pat.pk0012.f3	AA495712	Complement C7
pat.pk0019.e12	AI980259	Killer cell inhibitory receptor-like protein
pat.pk0020.d10	AI980295	Granzyme B precursor
pat.pk0020.e6	AI980307	CEPU-1 protein precursor
pat.pk0020.e7	AI980308	Common cytokine receptor γ
pat.pk0021.c12	AI980335	Coatomer ε subunit
pat.pk0023.e4.f	AI980442	Zinc finger protein
pat.pk0026.f7.f	AI980563	GA17 protein
pat.pk0024.h9.f	AI980521	Ubiquitin carboxyl-terminal hydrolase UNP
pat.pk0025.d2.f		Erythropoietin receptor
pat.pk0038.d7.f	AI980851	4-1BB ligand receptor precursor
pat.pk0039.a8.f	AI980983	Interferon-induced guanylate binding protein
pat.pk0049.c2.f	AI981244	Nuclear protein skip
pat.pk0049.d3.f	AI981250	NK-KB p50 subunit
pat.pk0050.h9.f	AI981335	Paxillin
pat.pk0051.b2.f	AI981344	Rearranged T-cell receptor δ-chain
pat.pk0052.a5.f		Eukaryotic translation initiation factor 3
pat.pk0052.b3.f	AI981384	Mitogen inducible gene-2
pat.pk0052.e4.f		Stress-induced phosphoprotein 1
pat.pk0062.h1.f	AI981685	Fas receptor
pat.pk0071.f9.f	AI982017	Receptor tyrosine kinase eph
pat.pk0078.a4.f	AI982241	Cell division protein kinase 7
pat.pk0078.h4.f	AI982258	P2Y purinoceptor 5
pmp1c.pk005.k10		Granulocyte colony-stimulating factor receptor precursor
pmp1c.pk005.b24		Tapasin 1 homolog
pmp1c.pk005.h10		MD-1 protein
pmp1c.pk005.h12		Caspase-1
pmp1c.pk005.h22		Toll-like receptor
pmp1c.pk006.k15		BCL2
pmp1c.pk007.e22		Stomatin
pmp1c.pk007.m18		MHC class II β-1 and β–2 domains
Identical genes repressed in secondary infection		
pat.pk0021.g4	AI980368	Antigen peptide transporter 1
	X56772	Osteopontin

comparable changes when comparing *E. acervulina* with *E. maxima.*

Discussion

Eimeria protozoa invade, grow, and reproduce within intestinal epithelial cells of the chicken. In response, the host produces a cellular immune response mediated primarily by local IELs (Bhogal et al. 1989; Lillehoj and Trout 1996; Lillehoj 1998). Intestinal IELs may or may not limit the extent of parasitism depending on a variety of factors including, but not limited to, the species of *Eimeria*, the infective dose, chicken nutritional status, and environmental stress. As a step toward understanding the complex immune responses resulting from parasite infection, we used cDNA microarrays to profile changes in gene expression in intestinal IELs of *Eimeria*-infected chickens. In addition, we searched for commonalities in three different parameters: *E. acervulina* vs. *E. maxima*, primary vs. secondary infection, and days post-infection. Both species of *Eimeria* are found commonly in poultry farming and invade different regions of the small intestine (Conway and McKenzie 1991).

In total, we observed up-regulation of 42 chicken gene transcripts and repression of 45 transcripts at days 1–4 following primary or secondary infection with *E. acervulina* or *E. maxima*. Generally, primary infection

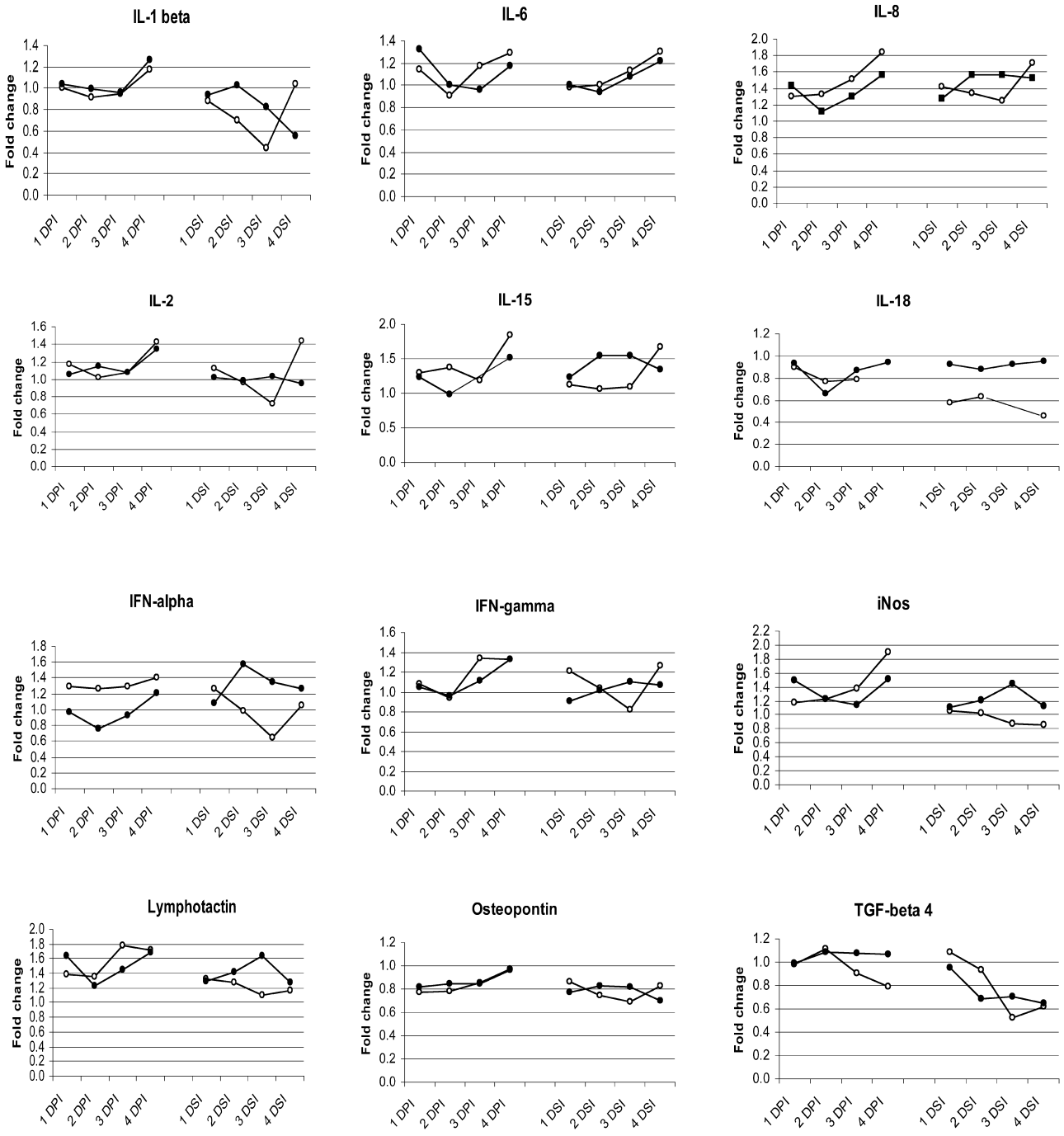

Fig. 2 Changes in cytokine transcript levels following *Eimeria* infection. ○ *E. acervulina* infection, ● *E. maxima* infection. *DPI* Days post-primary infection, *DSI* days post-secondary infection. Fold changes in transcript levels are expressed as the $\log_2$ ratio and were analyzed using SAM software. GenBank accession numbers are: IL-1β (Y15006), IFN-α (U07868), lymphotactin (AF006742), iNOS (D85422), IL-2 (AF017645), osteopontin (X56772), IL-18 (AJ277865), IL-6 (AJ309540), IL-8 (AI980458), IL-15 (AF139097), TGF-β4 (M31160), and IFN- γ (U27465). No data were obtained at 3 DPI with *E. maxima* for IL-15 and at 4 DPI and 3 DSI with *E. acervulina* for IL-18

induced more changes in gene expression than did secondary infection, and *E. acervulina* caused more changes than *E. maxima*. Of the 42 activated transcripts, five were common among the three parameters analyzed, and of the 45 repressed transcripts, eight were shared. These 13 gene transcripts may encode proteins involved in intestinal anti-parasite immunity since similarities in immune responses among different *Eimeria* species are well-documented (Lillehoj 1998; Lillehoj and Lillehoj 2000). In this regard, it is interesting to note that some of these transcripts are encoded by immune-associated genes. As many *Eimeria*-regulated genes do not have

defined functions in the infection process, these results provide the foundation for future studies to clarify unknown aspects of the early cellular immune mechanisms following parasite infection.

With regard to expression profile of cytokine genes, previous studies have shown that chicken interferons and interleukins play an important role in the cell-mediated immune response to *Eimeria*. For example, significantly increased interferon (IFN)-γ mRNA was detected in intestinal cells following infection with *E. tenella* or *E. maxima* (Laurent et al. 2001; Yun et al. 2000b). Results of our DNA microarray analysis, in which levels of IFN-γ mRNA increased on days 3 and 4 following primary infection with *E. acervulina* or *E. maxima*, supports these findings . Additionally, we observed previously that duodenal IELs obtained from chickens immunized simultaneously with a coccidia DNA vaccine and IL-8 or IL-15 had significantly increased numbers of $CD3^+$ T-cells (Min et al. 2001). Similarly, the number of $CD3^+$ cells was increased in the jejunal mucosa of chicken infected with *E. maxima* (Laurent et al. 2001). These findings suggest a relationship among *Eimeria* infection, IL-15, and $CD3^+$ cells. Our microarray data support this conclusion by demonstrating that levels of IL-15 transcripts were upregulated at all time points examined following primary or secondary infection with *E. acervulina* or *E. maxima*.

In conclusion, this study provides the first description of global transcriptional responses elicited in chicken intestinal lymphocytes following *Eimeria* infection. Our findings clearly indicate that *E. acervulina* and *E. maxima* induce similar genetic changes and that the levels of several transcripts were commonly affected in response to both *Eimeria* species. Furthermore, the expression patterns of cytokine genes followed similar trends after experimental infection. Additional microarray studies with other *Eimeria* species will provide enhanced insights into the host protective responses against oral infection with *Eimeria*.

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