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Invited review

Cell: sporozoite interactions and invasion by apicomplexan parasites of the genus *Eimeria*

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Abstract

The site specificity that avian *Eimeria* sporozoites and, to a more limited degree, other apicomplexan parasites exhibit for invasion in vivo suggests that specific interactions between the sporozoites and the target host cells may mediate the invasion process. Although sporozoite motility and structural and secreted antigens appear to provide the mechanisms for propelling the sporozoite into the host cell, there is a growing body of evidence that the host cell provides characteristics by which the sporozoites recognise and interact with the host cell as a prelude to invasion. Molecules on the surface of cells in the intestinal epithelium, that act as receptor or recognition sites for sporozoite invasion, may be included among these characteristics. The existence of receptor molecules for invasion by apicomplexan parasites was suggested by in vitro studies in which parasite invasion was inhibited in cultured cells that were treated with a variety of substances designed to selectively alter the host cell membrane. These substance included cationic compounds or molecules, enzymes that cleave specific linkages, protease inhibitors, monoclonal antibodies, etc. More specific evidence for the presence of receptors was provided by the binding of parasite antigens to specific host cell surface molecules. Analyses of host cells have implicated 22, 31, and 37 kDa antigens, surface membrane glycoconjugates, conserved epitopes of host cells and sporozoites, etc., but no treatment that perturbs these putative receptors has completely inhibited invasion of the cells by parasites. Regardless of the mechanism, sporozoites of the avian *Eimeria* also invade the same specific sites in foreign host birds that they invade in the natural host. Thus, site specificity for invasion may be a response to characteristics of the intestine that are shared by a number of hosts rather than to a unique trait of the natural host. Protective immunity elicited against avian *Eimeria* species is not manifested in a total blockade of parasite invasion. In fact, the effect of immunity on invasion differs according to the eliciting species and depends upon the area of the intestine that is invaded. Immunity produced against caecal species of avian *Eimeria*, for example *Eimeria tenella* and *Eimeria adenoeides*, inhibits subsequent invasion by homologous or heterologous challenge species, regardless of the area of the intestine that the challenge species invade. Conversely, in birds immunised with upper intestinal species, *Eimeria acervulina* and *Eimeria meleagrimitis*, invasion by challenge species is not decreased and often is significantly increased.

Keywords: Apicomplexa; Avian *Eimeria*; Sporozoites; Cell invasion; Receptor molecules; Immunity

1. Introduction

The Apicomplexa are a large group of parasites that include causative agents of serious human and agricultural diseases such as malaria, toxoplasmosis, coccidiosis, cryptosporidiosis, etc. The first step in the disease process in the host animal is the invasion of susceptible cells by the parasites. Despite the importance of cell invasion, both in the initiation of the infection and as an attractive target for control intervention, many of the events that are involved in the process have not been completely defined. The obligate intracellular nature of the parasites, plus the facts that sporozoites and host cells carry out many of the same biochemical processes and that invasion in birds occurs in the complex environment of the intestine present a daunting task to isolate and characterise those processes involved solely in the entry of the parasite into the host cell.

In the last 20 years, major strides have been made in defining the contributions of apicomplexan parasites to the process of cell invasion, many of which appear to be conserved across the phylum (Dolbrowolski and Sibley, 1996; King and Bruce, 1997; Dubremetz et al., 1998). For example, the invasive stages of apicomplexan parasites are characterised by a unique complex of specialised structures and membrane-bound organelles. This complex is located in the anterior end of the parasite and secretions from the organelles are believed to be essential for the

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recognition, attachment, and invasion of target host cells. Earlier, it was proposed that internalisation of these parasites occurred through passive phagocytosis by the host cell (Doran and Vetterling, 1967; Doran, 1982). More recently, that hypothesis has been replaced by one that presumes active participation on the part of the parasite (Russell and Sinden, 1981; Nichols et al., 1983; Dolbrowolski and Sibley, 1996). Sporozoite motility is a primary requisite for invasion of cells (Russell and Sinden, 1981; Preston and King, 1992). Sporozoites 'recognise', contact, and finally enter the host cell through a circular gliding motion that is produced by the capping of surface membrane proteins toward the rear of the parasite. This activity is required for invasion both in the host animal and in cell culture. In vivo, sporozoites emerging from the oocyst within the intestine of the host bird migrate through the intestinal lumen to make contact with the columnar epithelial cells for invasion to occur. Once contact has been made, the sporozoite must then propel itself into the host cell. In vitro, gravity provides the impetus for arrival at the host cell because sporozoites are usually inoculated onto cell cultures that are grown in a horizontal position. However, motility is still required, as it is in vivo, for penetration of the host cell.

Once the sporozoite attaches to the host cell, invasion continues with the invagination of the host cell membrane in front of the advancing parasite, and ends with the sealing off of the membrane at the site of parasite entry. Internalisation appears to be non-destructive to the host cell plasma membrane. Sheetz et al. (1976) offered a model for the invagination of membranes that involved the insertion of an amphipathic molecule into the cytosolic layer of the plasma membrane, causing it to expand relative to the outer layer. Evidence for secretion of materials by *Eimeria* sporozoites when inoculated into cell cultures or exposed to cell-conditioned medium suggests that the model might apply to cell invasion.

Although the host cell is believed to play little role in the physical propulsion of the parasite into the host cell, the importance of the appropriate host cell as a source of surface molecules that may serve as receptors or of metabolic products that attract or activate the parasite has become recognised. Recently, the activities of the apicomplexan invasive stages in the invasion process have been reviewed in depth (Tomley et al., 1997; Dubremetz et al., 1998) and will be discussed only to clarify or expand other points. The current review will cover primarily the contributions of the host animal or cell to the invasion process. The emphasis will be on: (1) factors that may contribute to site or host cell specificity for invasion; and (2) the effect of prior exposure of the host animal (immunity) or cultured cells to sporozoites on subsequent invasion of the cells by challenge species. The bulk of the discussion will concern sporozoites of avian *Eimeria* species, but will be supplemented with information that is known about other apicomplexan parasites.

2. Host and site specificity for invasion in vivo

In vivo, the avian *Eimeria* exhibit a high degree of host specificity for development as defined by the ability to complete the life cycle and produce infection. With few recognised exceptions, the avian *Eimeria* are limited to a single host species as well as to selective organ systems, portions of these organ systems, types of cells and locations within the cells (Marquardt, 1973; Vetterling, 1976). In contrast, the invasive stages of the *Eimeria* exhibit a far lower degree of host specificity for invasion and will excyst and infect intestinal cells in a number of avian and mammalian hosts (Doran, 1982). Although not host specific for invasion, the avian *Eimeria* exhibit a high degree of site specificity with most species invading narrowly defined areas within the intestine of the host birds (Long and Millard, 1976; Joyner, 1982; Augustine and Danforth, 1986). Site specificity for invasion occurs not only in the natural host but also in foreign host birds (Table 1; Augustine and Danforth, 1990). Species of coccidia that preferentially invade the lower intestine of the natural host also preferentially invade the same area in a foreign host bird. For example, *Eimeria tenella*, the caecal coccidium of chickens, invades the caeca of turkeys as effectively as it invades the caeca of chickens (Augustine and Danforth, 1990). Similarly, species of coccidia that invade the upper intestine of the natural host, do so in the foreign host (Augustine and Danforth, 1990). Site specificity for invasion is so rigorous that intravenous, intramuscular, or intraperitoneal injections of mammalian or avian *Eimeria* result in infections in the same area of the intestine as would be expected if the animals had been given the parasites orally (Joyner, 1982). The qualities of each area of the intestine that promote invasion by one species of *Eimeria* and not by

Table 1
Invasion in natural and foreign host birds

Eimeria Species	Natural host	Area of intestine[a]	Sporozoites/cross-section of intestine in	
			Chickens	Turkeys
E. tenella	Chicken	I–C juncture	<1	<1
		Caecal neck	3	1.5
		Caecal pouch	99	100
E. adenoeides	Turkey	I–C juncture	14	877
		Caecal neck	6	438
		Caecal pouch	<1	<1
E. acervulina	Chicken	Upper DL	129	519
		Mid DL	134	545
		Lower DL	129	419
E. meleagrimitis	Turkey	Mid SI	5	43
		(Three areas)[b]	6	49
			4	34

[a] I–C = ileocaecal juncture; DL = duodenal loop; SI = small intestine; YSD = yolk stalk diverticulum.

[b] Three equidistant areas between the insertion of the bile ducts at base of duodenal loop and the yolk stalk diverticulum.

another are unknown at this time, although several hypotheses have been offered. One hypothesis attributes site specificity to the length of time required for emergence of the sporozoite from the oocyst, e.g. species requiring the longest time for excystation to occur tend to invade the lowest sites in the intestine (Farr and Doran, 1962). After quantifying the distribution of oocysts, sporocysts and sporozoites of several species of avian *Eimeria* in the digestive tract and faeces, Shiotani et al. (1992) hypothesised that site specificity for invasion by each species is determined before invasion takes place. Other proposals suggest that the relative distribution of host cell surface molecules along the length of the intestine produces an affinity for one area of the intestine over another.

3. Affinity for specific cells in vitro

Although site specificity for invasion cannot be studied as such in vitro, differences in invasion of cell cultures prepared from tissues from a variety of sources suggest that some cells are more permissive for invasion by each *Eimeria* species than others (Doran and Augustine, 1977; Augustine, 2000 (in press)). Sporozoites of *Eimeria* species differ widely in their ability to invade different cell types in vitro and the differences in invasion appear to be due to characteristics of both the host cell and the sporozoite (Augustine, 2000 (in press)). For example, with *E. tenella*and *Eimeria adenoeides* sporozoites,invasion in an established cell line (baby hamster kidney) and in a culture of turkey caecal cells that had undergone seven passages in vitro was significantly greater than invasion in two primary avian kidney cell preparations (Table 2). Moreover, within a given cell type, e.g. baby hamster kidney cells, invasion by *E. adenoeides* was constantly and significantly greater than invasion by several other species or isolates that were tested, and invasion by *Eimeria acervulina* was consistently and significantly lower. The differences in invasion did not appear to be due either to the drug sensitivity profile of the isolate or to the length of time the isolate had been passaged in the laboratory (Augustine, 2000 (in press)). Similarly, cell preference for invasion in vitro did not appear to be related to either the host animal or specific tissue from which the cultures were derived. *Eimeria tenella* invaded cell cultures prepared from the kidneys of five non-host gallinaceous birds in the same numbers that they invaded cultures of chick kidney cells (Doran and Augustine, 1973; Doran, 1982). This correlates well with the lack of host specificity for invasion observed in vivo (Augustine, 1986). In addition, there is little preference for cultures of intestinal cells over cell cultures prepared from other organs (Augustine, 1994; Augustine, 2000 (in press)). Turkey kidney cell cultures were as permissive for invasion by the turkey caecal coccidium, *E. adenoeides*, as were turkey caecal cell cultures (Augustine, 1994). Superficially, these data suggest that at least within the avian species of *Eimeria*, factors that influence site specificity for invasion in vivo may not be expressed in vitro. However, one cannot rule out the possibility that cells that have undergone multiple passages in vitro may have changed to the point that they no longer express the factors responsible for site selection or that the particular cells which produced the factors failed to grow in culture.

Alternatively, site specificity for invasion by each species may be associated with unique conditions in the intestinal lumen such as pH, enzyme makeup, mucous, cell metabolites, etc. There are at least two studies that support the hypothesis that cell metabolic products may be involved in the invasion process and, in some cases, may promote invasion by one species but not by others. In the first, release

Table 2
Invasion of baby hamster kidney (BHK), chick caecal (CC), primary chick kidney (PCK), and primary turkey kidney (PTK) cell cultures by *Eimeria tenella* and *E. adenoeides* sporozoites (SZ)

	Experiment 1		Experiment 2		Experiment 3	
Cell Type	Confluency[a]	SZ/mm^2 cells	Confluency[a]	SZ/mm^2 cells	Confluency[a]	SZ/mm^2 cells
E. tenella						
BHK	15 ± 1^{b}	$218 \pm 29^{b,c}$	19 ± 5^{c}	284 ± 72^{b}	31 ± 1^{b}	299 ± 51^{b}
CC	10 ± 1^{c}	$231 \pm 29^{b,c}$	22 ± 1^{c}	233 ± 22^{b}	31 ± 1^{b}	$266 \pm 30^{b,c}$
PCK	28 ± 4^{b}	136 ± 17^{c}	46 ± 4^{b}	55 ± 3^{c}	31 ± 1^{b}	137 ± 7^{c}
PTK	59 ± 5^{b}	184 ± 23^{c}				
E. adenoeides						
BHK	16 ± 4^{d}	94 ± 10^{b}	31 ± 1^{b}	62 ± 5^{c}		
CC	10 ± 1^{d}	107 ± 8^{b}	32 ± 1^{b}	86 ± 4^{b}		
PCK	34 ± 8^{c}	58 ± 8^{c}	31 ± 1^{b}	48 ± 7^{c}		
PTK	61 ± 3^{b}	$80 \pm 10^{b,c}$				

[a] Confluency = percentage of coverslip covered by cells.
[b–d] Data expressed as means ± SEM of three cover slips; within experiments, parasites, and parameters, means followed by unlike superscripts differ significantly ($P < 0.05$).

of sporozoite molecules believed to be involved in the attachment of *E. tenella* to host cells was induced by conditioned medium from uninfected MDBK cell cultures (Bumstead and Tomley, 1997). The release was induced by the binding of host cell molecules to the surface of the parasite, presumably via specific receptors (Tomley et al., 1997). In the second study, conditioned medium from cultures of intestinal and caecal cells of both chickens and turkeys enhanced invasion of cells by sporozoites of turkey coccidia. Similar enhancement was not realised with conditioned medium from cultures of several other avian and mammalian primary and immortalised cells (Augustine and Jenkins, 1998). Thus, soluble products of host cells may have acted upon the cell itself, the sporozoite, or both, and increased either the attraction between the participants or the permissiveness of the cells for invasion. There was no report of chemotactic attraction in either experiment. However, the initial presentation of the sporozoites with respect to the host cells, the extracellular environment and gradients of host cell metabolites differ markedly between the intestinal and cell culture milieus. Thus, cellular influences on site specificity for invasion in vivo may be present but not detectable in vitro.

4. Is invasion mediated through host cell receptors?

In numerous experiments, treatment of cultured cells with a variety of classes of exogenous compounds including enzymes, cationic complexes or molecules, protease inhibitors, parasite homogenates or monoclonal antibodies (MAb) significantly altered invasion of the cells by *Eimeria* sporozoites (Fayer, 1971; Augustine, 1985a,b, 1986, 2000; Augustine and Danforth, 1984, 1987; Kogut and Lange, 1988; Fuller and McDougald, 1990; Crane and McGaley, 1991). Because these treatments were designed to selectively remove, bind, protect or neutralise cell surface moieties, it was hypothesised that they changed the cell surface profile or disorganised the membrane lipid bilayer, thereby limiting the host cell-parasite interactions that culminate in invasion (Augustine, 2000). While the effects of some of these treatments on invasion were similar for all species, others varied according to the species of *Eimeria*-being tested. That there were differences in effects on invasion suggests that there are both conserved and unique aspects of the invasion process among species of avian *Eimeria* and that a host cell surface feature may provide an attractive target for invasion. Similar observations have been made for other apicomplexan parasites. For example, extensive treatment of host cells with enzymes enhanced invasion by *Toxoplasma gondii* (Lyke et al., 1965; 1975). In addition, polycationic compounds produced morphological changes in a variety of types of host cells and significantly enhanced invasion by *T. gondii*. The morphological changes in the host cells were similar to those produced by the 'penetration enhancing factor' secreted by *T. gondii* (Werk et al., 1984). Later studies showed that a peptide that inhibits binding of laminin to the laminin-binding protein on host cells reduced parasite attachment to the cells (Furtado et al., 1992b). Collectively, the data show that modification of the host cell with a variety of compounds significantly altered the ability of apicomplexan sporozoites to invade the treated cells, suggesting the presence of a host cell surface receptor molecule or recognition site for invasion. It must be remembered, however, that the incubation of cells in the treatment compounds causes not only the specific effect, e.g. removal, blocking or neutralisation of surface moieties, but also more general movement or aggregation of proteins within the plane of the membrane. Effects of some of the cell treatments may be attributed to this non-specific activity, although the author is not aware of published studies that correlate general membrane disorganisation with invasion by apicomplexan parasites.

More solid evidence for the participation of specific receptor or recognition molecules in interactions between sporozoites of *Eimeria* species and host cells was first presented by Augustine (1989). The receptor hypothesis was based upon studies in which electrophoretically-separated antigens of *Eimeria* sporozoites were bound by components of a homogenate of host cells. A Mab developed against the most intensely bound sporozoite antigen (40 kDa) significantly inhibited invasion of cells by sporozoites of several *Eimeria* species (Augustine, 1989, 1991). The host cell molecules binding to the sporozoite antigens were proposed to be receptors for invasion.

5. Potential host cell receptor molecules for *Eimeria* species

Membrane glycoconjugates have been identified as potential host cell receptors for invasion. In many parasite-host cell interactions, including those between host cells and a number of intestinal protozoan parasites, it has been proposed that attachment is mediated by lectin–ligand binding. The hypothesis is supported by the work of Alroy et al. (1989); Suprasert and Fujioka (1988) who reported marked differences in the distribution of carbohydrate residues on the luminal surface of the intestinal epithelium in the small and large intestines and caeca of chickens. It was further proposed that these interactions may play a role in site specificity for invasion by *Eimeria* sporozoites. Strout et al. (1994) described lectins that were part of the biochemical make-up of three species of avian *Eimeria*, *E. tenella, E. acervulina,*and *Eimeria maxima*. The lectin of each species had a different sugar affinity and was found almost exclusively in the sporozoites. Furthermore, there were marked correlations among the sporozoite lectins, the carbohydrate moieties of the intestinal cell surface, the pH optima of the lectin-carbohydrate interactions, the area of the intestine that was invaded by each species. Compelling arguments

supporting the proposal that lectins aid in site selection by the *Eimeria* are the facts that: (1) the hemagglutination reaction produced by the *E. tenella* lectin was neutralised by L-fucose which is found in large quantities in the caeca; and (2) fetuin, which also inhibited erythrocyte agglutination by *E. tenella* lectin, significantly inhibited invasion of cultured cells by *E. tenella* sporozoites (Strout et al., 1994). Augustine (1985b) reported that several lectins bound to the surface of cultured primary turkey cells, but except for wheat germ agglutinin, failed to inhibit invasion by several species of *Eimeria*. The action of wheat germ agglutinin was subsequently ascribed to binding of the lectin to anionic moieties of the host cell rather than to its specific sugar ligand, *N*-acetyl glucosamine. Conversely, Baba et al. (1996) proposed that the lectin and carbohydrate locations were reversed, with the lectin on the host cell surface and the carbohydrate associated with the sporozoites. The conclusions were based on data that showed that pretreating *E. tenella* sporozoites with a D-galactose-binding lectin or chick kidney cells with D-galactose significantly reduced invasion by the sporozoites.

Host cell membrane glycoconjugates could also be potential receptors for parasite molecules other than lectins. Microneme proteins of several apicomplexan genera including *Plasmodium*, *Eimeria*, *Toxoplasma* and *Sarcocystis*, contain conserved regions that may act as attachment points between the parasite and the host cell (Tomley et al., 1997; Dubremetz et al., 1998). One of these proteins, thrombospondin, usually binds to sulfated glycoconjugates on cell surfaces. The irregular distribution of these gycoconjugates in different tissues suggests a potential contribution to site selection for invasion.

Common epitopes of the intestinal epithelium and the sporozoite are also plausible candidates for receptors for invasion and contributors to site specificity. Common epitopes were demonstrated with Mab on the surface membranes of *E. tenella* sporozoites and the caecal epithelium of chickens, the target area for invasion by *E. tenella* (Vervelde et al., 1993). The authors proposed that the epitopes might serve as recognition molecules for invasion. This hypothesis was supported by similar labelling of caecal cells in turkeys, which *E. tenella* also invades, and the lack of labelling of cells in other areas of the intestine in either breed of bird (Vervelde et al., 1993). Interestingly, a similar phenomenon was observed in vitro. Monoclonal antibody 1209 was generated against refractile body antigens of *E. acervulina* but cross-reacted with refractile bodies of all avian *Eimeria*that have been tested, refractile bodies of *Caryospora bigenetica*, and with epitopes on the surface of cultured baby hamster kidney cells (Augustine, 1999, 2000 (in press)). Treatment of baby hamster kidney cells with this MAb before inoculation with sporozoites significantly reduced the ability of the sporozoites to invade the cells (Augustine, 1999). The inhibition of invasion occurred only with apicomplexan parasites that shared the epitope (Augustine, 2000 (in press)), indicating that the inhibitory activity was a specific antigen-antibody interaction and not a non-specific reaction of the MAb with Fc receptors on the host cell.

6. Potential host cell receptors for other apicomplexan parasites

Although not as well documented, site specificity for invasion has also been described for other apicomplexan sporozoites. For example, in studies with suckling mice, putative host cell receptors for a purified sporozoite membrane-associated protein of *Cryptosporidium parvum*, CP47, were identified in higher concentration in ileal than duodenal tissues, which may explain the affinity of *C. parvum* for the ileum (Nesterenko et al., 1999). Both the binding of host cell and parasite proteins and invasion of cultured cells by live *C. parvum* sporozoites were inhibited by manganese. The activity of manganese appeared to be expressed at the level of the host cell (Nesterenko et al., 1997). Only low amounts of the host cell receptor were detected in the ileum of adult mice, suggesting that developmentally regulated antigens may be involved in the invasion process (Nesterenko et al., 1999).

7. Are receptors the total picture?

The evidence for reciprocal receptor molecules for host cells and apicomplexan parasites is strong, but it is unlikely that host cell receptors exist solely for invasion of cells by parasites. It is more likely that features that are recognised as receptors for parasite invasion have other biological functions that are critical for the survival and growth of the host cell. However, preference for one cell type or area of the intestine over another may still be influenced by the relative distribution of a particular molecule within the host or among different types of cultured cells, even if its primary biological function has little to do with parasite invasion. Several apicomplexan parasites invade a wide variety of cells in vitro (Doran, 1982; Dubremetz et al., 1998) suggesting that if host cell receptors are involved in cell invasion, then they are widely distributed in nature. There is also evidence that expression of receptor molecules, and therefore permissiveness of host cells for parasite invasion, may vary with the stage of the host cell cycle (Grimwood et al., 1996) and possibly, with the polarity of the cells (Furtado et al., 1992a). For example, *T. gondii* attachment to several cell types increased markedly as the cells proceeded through the G1 phase to the mid-S phase and then decreased as the cells entered the G2-M boundary, suggesting that the parasite binds specifically to host cell molecules upregulated in the mid-S phase of the cell cycle (Grimwood et al., 1996). Host cell receptor molecules maybe only one of many forces in the attachment and internalisation of apicomplexan parasites. Other characteristics such as membrane fluidity and

cytoskeletal composition may exert strong influence on the invasion process.

8. The effect of immunity on invasion

Host animals show varying degrees of protective immunity to the coccidia ranging from complete innate immunity to foreign coccidia (host specificity) to active immunity acquired as a result infection (Rose, 1982). Although the literature contains an abundance of contradictory reports on the effects of immunity on invasion, it is now generally agreed that the protection afforded by either innate or acquired immunity is not mediated through a total blockade of sporozoite invasion (Rose, 1982). In fact, the effect of immunity on invasion varies greatly among avian *Eimeria* species and appears to be highly dependent upon the area of the intestine in which the immunising species invades (Augustine and Danforth, 1986). Species such as *E. tenella* and *E. adenoeides* that invade the lower intestine and caeca of chickens and turkeys, respectively, elicit an immune response that reduces invasion by homologous challenge organisms (Augustine and Danforth, 1990; Augustine et al, 1991; Augustine, 2000 (in press)). The reduction in invasion is usually around 50 to 65% as compared with invasion in non-immunised birds (Rose et al., 1984; Augustine and Danforth, 1986). Inhibition of invasion in the immune host is apparently a non-specific phenomenon because immunity to one species of *Eimeria* produces almost as great a reduction in invasion by heterologous challenge organisms as it does with a homologous challenge (Augustine and Danforth, 1990; Augustine et al, 1991; Augustine, 1996). The inhibition occurs regardless of the site of invasion of the challenge species in the intestine. For example, immunisation of chickens with *E. tenella*, the caecal coccidium of chickens, significantly decreased (41 to 51%) invasion by *E. acervulina*, which primarily invades the upper jejunum and duodenal loop (Augustine, 1999). Because excystation of *E. tenella* was shown to be non-host specific and to be similar in immunised and non-immunised chickens, the decrease in numbers of intracellular sporozoites was probably caused by a factor in the lumen of the intestine of the immunised birds (Rose and Hesketh, 1987). Several hypotheses have been offered for the inhibition of invasion. One is that invasion of immunised chickens by *E. tenella* sporozoites was reduced by the intervention of secretory IgA antibodies and that a non-specific antisporozoite effect might be associated with the secretory IgA-mediated immune response (Davis et al., 1978; Rose and Hesketh, 1987). Another hypothesis is that a sporozoite-induced effect on the host cell rather than the immune response produced the effect on invasion (Augustine, 1996, 2000 (in press)). Inhibition of invasion, although a manifestation of the immune response, is probably not a critical element in the control of coccidial infection in the immune host (Rose and Hesketh, 1987). This conclusion is based on the observations that: (1) invasion in solidly protected birds is still substantial enough to produce clinical infection; (2) biliary IgA and its effect on numbers of recoverable sporozoites are transient in nature; and (3) whereas immune control of *Eimeria* infection is highly species specific, the influence of immunity on invasion appears to be less so. In contrast to the reduction in invasion produced by lower intestinal species of *Eimeria*, immunity elicited by species invading the mid and upper intestine of chickens and turkeys either had little effect on invasion or actually increased invasion by either a homologous or heterologous challenge species (Augustine and Danforth, 1985; Augustine, 1996).

Increases and decreases in numbers of intracellular sporozoites in intestinal cells have commonly been attributed to changes in invasion. This is probably correct. However, an alternate explanation is that invasion in immunised birds is similar to that in non-immunised birds but that the migration of the sporozoites from the intestinal cells to the areas where development occurs has been altered. *Eimeria* sporozoites have been shown to invade and exit cells shortly after invasion both in vivo and in vitro (Long and Speer, 1997; Doran, 1982). If alterations in the host cell cytoplasm or membrane occur as a result of prior exposure (or immunity) to sporozoites, then the ability of the challenge species to exit the cells could be modified. The numbers of sporozoites in these cells would give the impression of reduced or enhanced invasion when in fact it could be a function of the time spent in the cell which was invaded.

9. Effect of prior inoculation of cultured cells with *Eimeria* sporozoites on invasion by a second species

On a very simplistic level, repeated inoculation of cell cultures can be compared with immunisation of the host animal in that both the cultured and intestinal cells are exposed to sporozoite antigens for a period of time. The effect of previous inoculation of cultured cells with sporozoites (prior exposure) on subsequent invasion by challenge organisms is similar to that which occurs in the immunised animal. Cultured cells infected with lower intestinal species of *Eimeria* become less permissive for invasion by a second species, while those infected with an upper intestinal species of *Eimeria* become more permissive (Augustine, 1996, 2000 (in press)). The activity in cell cultures is more transitory than in birds, occurring within 4 days of initial infection but not at 5 days after infection (Augustine, 1996). Because prior exposure to sporozoites produced a similar effect on invasion in birds and cell cultures, the effect is more likely to have been precipitated by activity of the intracellular sporozoites or reaction of the host cell to the invasion process than by an element of the immune response.

The interaction between the apicomplexan sporozoites and host cells that culminates in successful invasion is an intricate, multifaceted series of events. The work that has been conducted presents convincing evidence that invasion

is regulated by associations between specific molecules of the sporozoites and host cells. Very simplistically, the sporozoite apparently possesses surface molecules and secreted antigens through which it contacts the host cell and induces its internalisation. The host cell offers a membrane site for sporozoite contact and may secrete soluble metabolites that enhance the probability of invasion of that particular cell. A number of physiologic conditions, including previous exposure to the parasites or immunity, can alter the permissiveness of the cells for invasion both in vivo and in vitro.

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