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journal or publication title	The journal of protozoology research
volume	13
number	3-4
page range	34-43
year	2003-10
URL	http://id.nii.ac.jp/1588/00001507/

DENDRITIC CELLS AND HOST RESISTANCE TO *TOXOPLASMA GONDII*

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ABSTRACT

Interferon gamma (IFN- γ)-dependent cell-mediated immunity plays a pivotal role in resistance against the development of toxoplasmosis, following *Toxoplasma gondii* (*T. gondii*) infection. There is now an increased recognition that this response is triggered by the critical initiation cytokine, IL-12 secreted by dendritic cells (DC), but not macrophages (M ϕ). This article reviews the current understanding of the role of DC during toxoplasmosis with a view to stimulate further research on the early DC activation events that may likely represent the "ignition switch" for type 1 cytokine responses leading to the control of *Toxoplasma gondii* infection.

KEYWORDS: *Toxoplasma gondii*, IL-12, IFN- γ , Dendritic cells, Host resistance, Mouse.

INTRODUCTION

The obligate intra-cellular protozoan parasite *Toxoplasma gondii* (*T. gondii*) can influence host resistance in a vast array of species of birds and mammals, including humans, by modulating immune functions in various cell types (Schade and Fischer, 2001; Alexander et al., 1997; Gazzinelli et al., 1993; Khan et al., 1994; Hunter et al., 1995; Yap et al., 2000). Usually asymptomatic in hosts with intact immunity, toxoplasmosis may lead to lethal damage when associated with immuno-suppressive states, for example AIDS, because of reactivation of encysted parasites, or when transmitted to the fetus during pregnancy (Frenkel, 1988; Remington et al., 1995). Although an effective live vaccine is available for animals (Buxton, 1993), such a vaccine is inappropriate for use in humans.

Several studies have provided evidence that protection against *T. gondii* parasites or other intra-cellular protozoan organisms not only depends on the initiation of specific immune responses but also strongly relies on the character of the response, that is, the Th1 – Th2 balance (Bourguin et al., 1998; Gazzinelli et al., 1991; Gazzinelli et al., 1993; Heufler et al., 1996; Khan et al., 1991; Khan et al., 1994a, b). The Th1 and Th2 cell subsets mediate separate effector functions, and the development of one population has important biologic consequences. The major mechanism by which immuno-competent hosts control *T. gondii* infection is considered to be cell-mediated immunity (Gazzinelli et al., 1993). The available evidence suggests that CD4+ protective cells belong to the Th1 subset (Gazzinelli et al., 1991; Gazzinelli et al., 1992). CD4+ cells are protective principally through interferon gamma (IFN- γ) production and can also activate CD8+ cells. CD8+ cytotoxicity (Khan et al., 1991; Khan et al., 1994a, b) aided by the helper activities of CD4+ cells (Araujo et al.,

1991) and the micro-bicidal or micro-biostatic activity of IFN- γ -activated macrophages (Suzuki et al., 1988) and non-phagocytic cells (Dimmier and Bout, 1993; Pfefferkorn and Guyre, 1984; Woodman et al., 1991) are the two major mechanisms of resistance to *T. gondii* infection. The physiologic regulation of Th phenotype development is still poorly understood, but because of the major histo-compatibility complex (MHC) restriction, attention in recent years has been focused on the major role of antigen presenting cells (APC) in the initiation of the immune response. In vitro studies have shown that activation of Th1 clones requires the presence of specialised and most potent APC, namely DC (Gajewski et al., 1991). DC, have recently been reported to promote the development of CD4+ Th1 cells through their production of IL-12 (Heufler et al., 1996; Macatonia et al., 1995), a heterodimer of 70 kDa consisting of two disulphide-bonded subunits, IL-12 p40 and IL-12 p35, which must be expressed in the same cell to generate bioactive IL-12. Production of IL-12 is elicited by microbial stimuli or during interaction with T cells via CD40 ligation (Gately et al., 1998; Trinchieri, 1998). Multiple evidence, indicates that *T. gondii* can trigger production of IL-12 in a T cell-dependent manner as shown in vivo in parallel studies (Reis e Sousa et al., 1997; Schulz et al., 2000) and on isolated APC, especially DC (Gazzinelli et al., 1994; Li et al., 1994; Reis e Sousa et al., 1997; Bliss et al., 1999; Fischer et al., 1999; 2000). Such a pathway has been implicated to be pivotal or the establishment of IFN- γ -dependent innate resistance (Sher et al., 1993; Suzuki et al, 1989). However, the parasite molecules and mechanisms involved are still unresolved to date and remain contentious as well as a subject for further research. The events showing the pathway for IL-12 dependent resistance in *T. gondii* infection are summarized in Figure 1.

DENDRITIC CELLS AND NOT MACROPHAGES FIRST SYNTHESIZE IL-12

Recent studies have demonstrated that DC, are the initial cells that synthesize IL-12 in the spleens of mice exposed in vivo to an extract of *T. gondii* (Sousa et al., 1997) and favours the development of Th1 cells from naïve CD4+ T cells (Heufler et al., 1996; Macatonia 1995). The production of IFN- γ by Natural killer (NK) cells early in the course of *T. gondii* infection results in the activation of M ϕ (Suzuki et al., 1988) and non-phagocytic cells, for example, fibroblasts (Pfefferkorn and Guyre, 1984), endothelial cells (Woodman et al., 1991), and enterocytes (Dimier and Bout, 1993) to kill parasites or inhibit parasite replication. This innate response affords protection to the host before the development of an adaptive immune response. DC cells could therefore play an important role not only in the initiation of adaptive immunity to *T. gondii* but also in the early induction of innate immunity to the parasite. After primary *T. gondii* infection, IL-12 produced by DC would be able to: activate NK cells to produce IFN- γ (Scharton-Kersten et al., 1996); promote T cell differentiation towards the Th1 phenotype, which produces IFN- γ (Heufler et al., 1996; Macatonia et al., 1995); and finally to synergize with the CD28-B7 interaction for efficient proliferation and production of IFN- γ by T cells (Murphy et al., 1994). In such a model, there is no need for a third cell, for example a M ϕ , to be involved in Th1 induction because the same APC that activate naïve microbe-specific T cells also dictate class selection as summarized in Figure 2. Furthermore, this model is consistent with the prediction that IL-12 production by M ϕ at the site of infection is unlikely to influence the differentiation of T helper cell precursors (Thp) taking place at a distance, in draining lymphoid tissues. Nonetheless, it can be seen in the same figure that IL-12 production by M ϕ may well be important in re-stimulating and maintaining the differentiated state of Th1 effectors at the site of infection.

Indeed, IL-12 and other cytokines (IL-1, TNF- α) are produced by M ϕ when exposed to live parasite or parasite extracts and stimulated NK cells to produce IFN- γ (Gazzinelli et al., 1993; Gazzinelli et al. 1994; Hunter et al., 1995; Sher et al., 1993). Rather, M ϕ -derived IL-12 is probably especially critical during the early innate phase of the immune response, before T cell clonal expansion takes place (Figure 2). However, because IFN- γ may be absolutely required to allow certain M ϕ populations to make IL-12 in response to pathogens, it is unclear how M ϕ production of the cytokine could ever be initiated. A possible explanation could be that NK cells, which serve as "surrogate" antigen-independent Th1 cells, are triggered to make IFN- γ in lymphoid tissues by IL-12 produced by DC arriving from the site of Infection (Figure 2). It is likely that this may be enough to ignite the IFN- γ cascade by providing sufficient IL-12 to trigger enough IFN- production to mediate M ϕ priming at the site of infection. The macrophage would then take over as the "furnace" for IL-12 production, driving IFN- γ -dependent host innate resistance (Figure 2). In this scenario, DC would be critical for initiating the innate as well as the adaptive immune response to infection. Which role *T. gondii* induction of IL-12 plays during the chronic stage of infection remains an issue of future research. A summary of cytokines and chemokines produced, their function and T helper responses induced by DC are summarized in Table 1.

Table 1. A summary of cytokines and chemokines produced, their function and T helper responses induced by dendritic cells (DC). Data were obtained from papers referenced in the text.

Cytokine Produced	Principal Activity	In vitro derived DC			Blood DC	
		CD14+ DC	CD34+ DC		Plasmacytoid DC	Myeloid DC
			CD1a+ DC	CD14+ DC		
IL-1	T cell stimulation	+	+	+		
IL-4	IgE isotype switch	-			-	
IL-6	T cell attraction	+	+	+	+	+
IL-7	T cell attraction		+	+	-	-
IL-8	Chemotactic factor	+			+	+
IL-10	Down regulates MHC II	+	-		+	+
IL-12	NK & CTL activation	+	+		+	+
IL-13	Inhibits inflammation		+	+		
IL-15	Chemo-attracts T cells		+	+	-	+
IL-18	Chemo-attracts T cells		+	+		
TNF- α	Increases MHC II		+	+	+	+
IFN- γ	Induction of MHC I & II					
TGF- β	DC signalling/differentiation		+	+		
GM-CSF	DC signalling/differentiation		+	+		
M-CSF	DC signalling/differentiation		+	+		
Chemokines Produced						
RANTES	T cell development	+	+	+	+	+
TECK	T cell development	+	+	+	+	+
MIP- α , β , γ	Chemo-attracts T cells	+	+	+	+	+
TRANCE	DC stimulation of T cells	+	+	+	+	+
ODF	DC stimulation of T cells	+	+	+	+	+
CD4+T cell response induced		Th1 and Th2		Th2?	Th1?	

Abbreviations: RANTES – regulated upon activation, normally t-expressed and presumably secreted; TECK – Thymus expressed chemokine; TRANCE – TNF-related activation induced cytokine; MIP – Macrophage inflammatory protein; ODF – Osteoclast differentiation factor; TNF – Tumor necrosis factor; IFN – Interferon; IL – Interleukin; M-CSF – Myeloid colony stimulating factor; TGF – Transforming growth factor; MHC – Major histo-compatibility complex; NK – Natural killer cells.

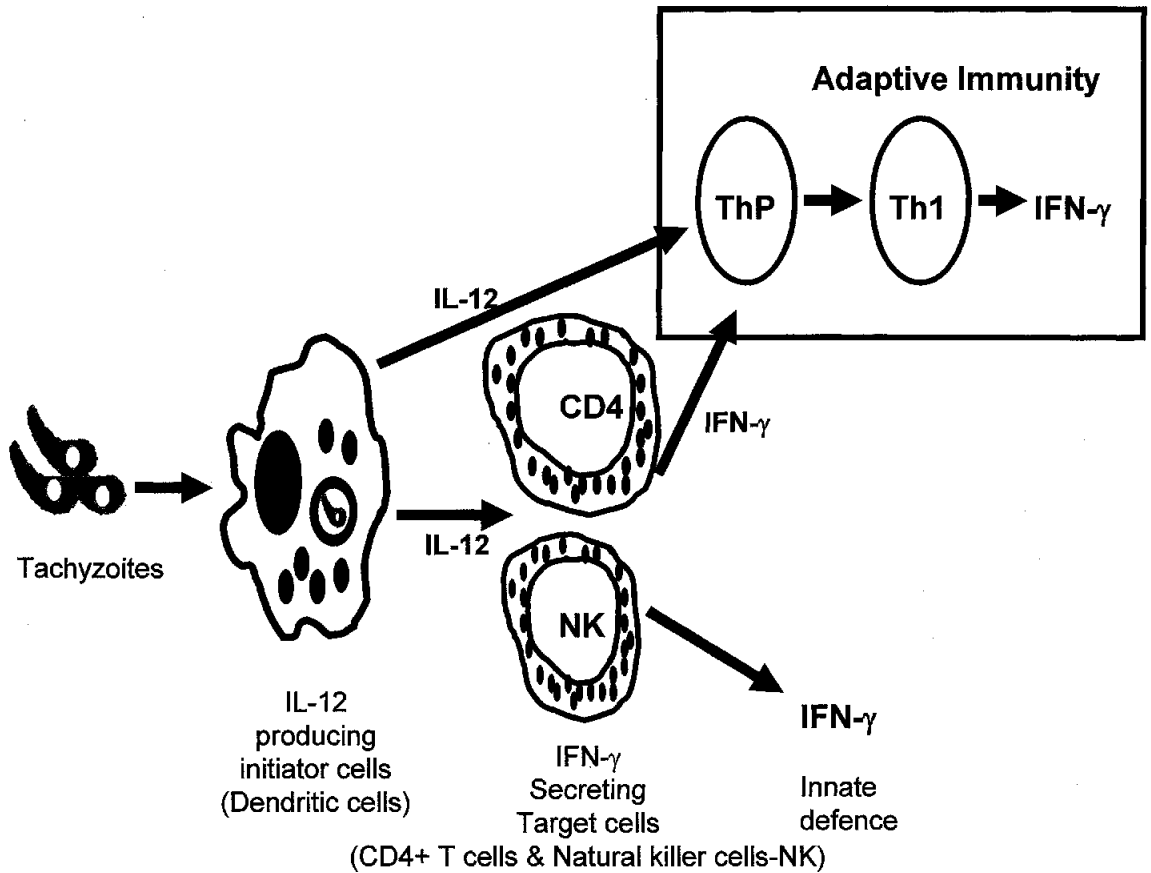


Figure 1. The current proposed pathway for IL-12 dependent resistance in *Toxoplasma gondii* infection.

Invading tachyzoites trigger IL-12 production from naïve initiator dendritic cells. The cytokine then triggers IFN- γ from un-primed natural killer cells and CD4+ T cells. The IFN- γ produced both protects the host from acute infection and together with IL-12 helps drive the development of an adaptive Th1 response, which prevents re-activation of the disease during the chronic stage.

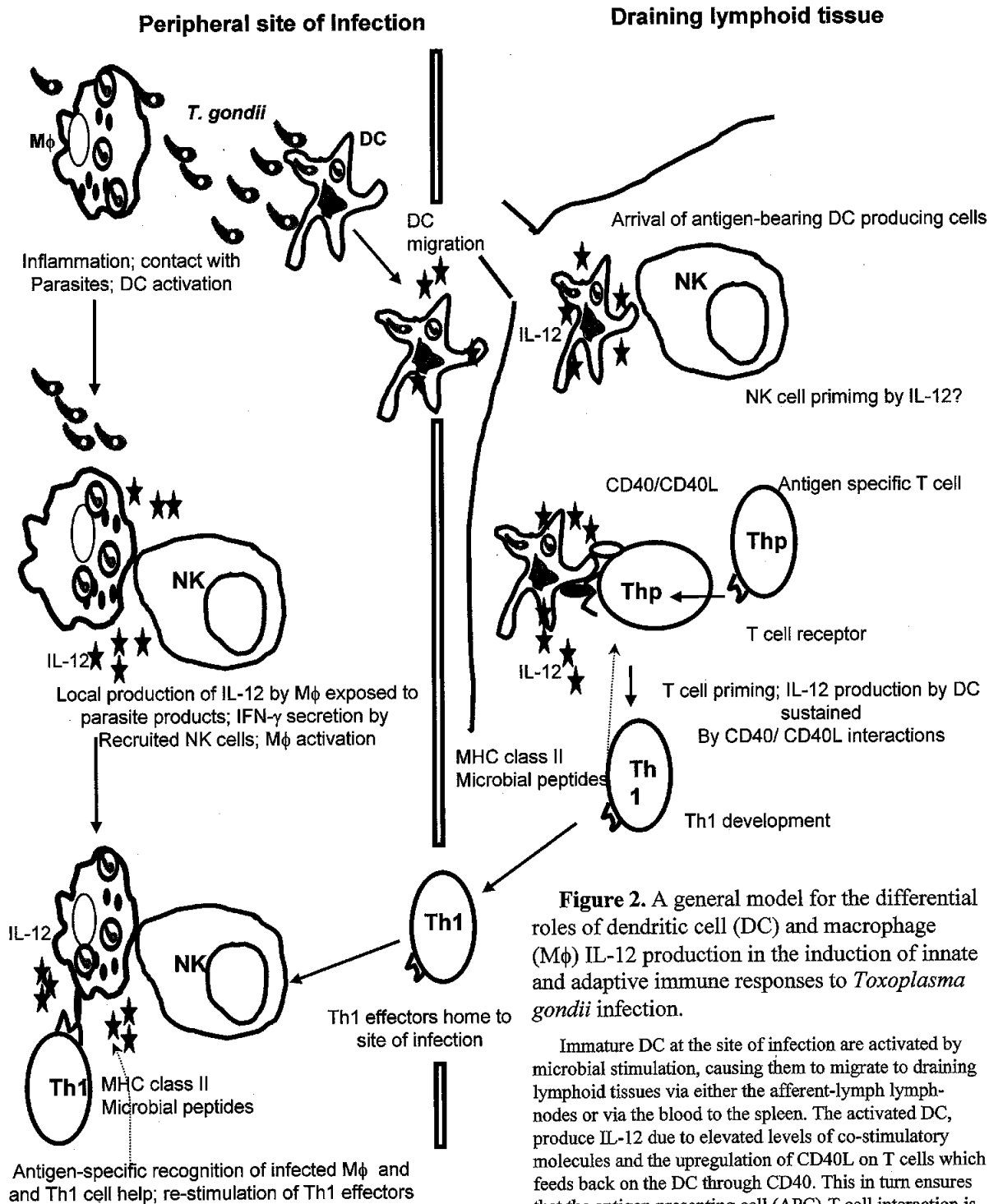


Figure 2. A general model for the differential roles of dendritic cell (DC) and macrophage (Mφ) IL-12 production in the induction of innate and adaptive immune responses to *Toxoplasma gondii* infection.

Immature DC at the site of infection are activated by microbial stimulation, causing them to migrate to draining lymphoid tissues via either the afferent-lymph lymph-nodes or via the blood to the spleen. The activated DC, produce IL-12 due to elevated levels of co-stimulatory molecules and the upregulation of CD40L on T cells which feeds back on the DC through CD40. This in turn ensures that the antigen presenting cell (APC)-T cell interaction is propitious for clonal expansion and TH1 differentiation of the responding T lymphocyte. Mφ-derived IL-12 may be critical in the early phase of immune responses before T cell clonal expansion has taken place. The Mφ-derived IL-12, then drives IFN-γ dependent host innate resistance.

TOXOPLASMA GONDII INDUCTION OF IL-12 IS ASSOCIATED WITH ACUTE VIRULENCE AND DEPENDS ON HOST GENOTYPE

By measuring M ϕ and DC production of cytokines, so far two parasite activities have been distinguished bio-chemically and functionally (Grunvald et al., 1996; Fischer et al., 1999), although the molecular mechanisms operating at this host-parasite interface is unclear. A recent study (Schade and Fischer, 2001) showed that the secretion of IL-12 p40 and the bioactive p70 heterodimer by inflammatory APC following exposure to live *Toxoplasma* or tachyzoite lysate is possible. In a series of experiments the authors showed that pre-digestion of *T. gondii* lysate with proteinase K abrogated its IL-12 inducing activity, thus indicating that a parasite protein or proteins triggers this response. Moreover, in the same study, the authors showed that, APC from various mouse-inbred strains showed a differential responsiveness, that is, cells from *T. gondii*-susceptible mice released more IL-12 upon toxoplastic challenge than those from resistant mice, although the infection rate and intracellular parasite growth were similar. In triggering APC production of IL-12, tachyzoites proved superior and more potent compared to bradyzoites prepared from the same *T. gondii* isolate. Furthermore, parasites of a mouse – virulent isolate became less efficient inducers of IL-12 following attenuation (Schade and Fischer, 2001). From these investigations, we can logically deduce that the parallel loss in APC stimulation in vitro and acute virulence in vivo suggest a linkage of both parasites capacities. Taken together, with the correlation on host side between the genotype-dependent mouse susceptibility to infection and cellular responsiveness to the parasite trigger, these findings indicate that an over production of parasite-induced IL-12 might represent a basic mechanism of *T. gondii* pathogenicity.

DENDRITIC CELLS ACT AS EFFECTOR CELLS DURING TOXOPLASMOSIS

These professional APC are the sentinels of the immune system, until recently no previous study had investigated the possibility of that DC may act as effector cells in this system, thereby constituting a micro-biostatic capacity. Thus, DC should be considered as cells involved in both induction and effector mechanisms of a specific immune response (Steinman and Nussenzweig, 1980). A recent study reported that DC, unlike neutrophils and lymphocytes, are highly permissive toward *T. gondii* invasion and replication (Channon et al., 2000). Other recent parallel investigations went further to test the ability of DC to act as effector cells in the immunological response (Aline et al., 2002). The authors demonstrated that IFN- γ -activation inhibits the replication of *T. gondii* in DC, with this inhibition being dose dependent. Moreover, in the same experiments it was shown that neither nitrogen derivatives nor tryptophan starvation appears to be involved in the inhibition of parasite replication by IFN- γ . Further experiments with oxygen scavengers indicated that intra-cellular *T. gondii* replication was oxygen dependent. Taken together, these results would suggest that in addition to their central role in the stimulation of immune responses, DC, probably may act as effector cells in the first line of defence against pathogen invasion.

OTHER ANTI-PARASITIC MECHANISMS FOR THE LYSIS OF INTRA-CELLULAR PROTOZOAN PARASITES

T. gondii has an unusually wide host spectrum, since almost all mammalian cells, including red blood cells, can be infected by this parasite in vitro (Werk, 1985). Although intracellular growth usually occurs within nucleated cells. Several studies have identified several antiparasitic mechanisms, including the production of

nitrogen monoxide (Marletta et al., 1988), which is responsible for the lysis of intracellular parasites in murine APC (Liew et al., 1990). This mechanism seems to be responsible for the inhibition of *T. gondii* replication in mouse APC pre-incubated with IFN- γ (Adams et al., 1990) but not in preincubated astrocytes (Halonen et al., 1998; 2000), endothelial cells, or enterocytes (Dimier and Bout, 1996; 1997; 1998). The second mechanism of inhibition related to IFN- γ -activated enterocyte has been shown to be due to a limitation of the availability of intracellular iron (Dimier and Bout, 1998), an element essential for metabolic processes. A third potential mechanism that has been sufficiently investigated involves the synthesis of toxic oxygen radicals (Aline et al., 2002). These intermediates seem to be responsible for the inhibition of *T. gondii* replication in immune murine APC (Murray and Cohn, 1979) but not in *in vivo*-activated APC (Sibley et al., 1985), endothelial cells (Woodman et al., 1991), or astrocytes (Halonen et al., 2000). The final anti-*T. gondii* mechanism is related to the production of an enzyme, 2,3-dioxygenase-indoleamine, which is synthesized in IFN- γ -activated human APC, retinal pigment cells, and fibroblasts (Murray et al., 1989; Pfefferkorn et al., 1984; Nagineni et al., 1996). This enzyme breaks down intracellular tryptophan, depriving the parasite of this amino acid, which is essential for its proliferation.

CONCLUDING REMARKS

To date many questions regarding the overall role of DC in the initiation of immune responses during infection with *T. gondii* (Johnson and Sayles, 1997) remain. It is well established that during per-oral infection this process is initiated locally in intestinal tissue (Frenkel, 1988). The big question is, are DC in the lamina propria and or Peyer's patches, themselves infected or do they acquire antigens shed by the parasite into the extracellular milieu? What is the nature of the parasite molecules responsible for DC activation? Do these molecules act directly on DC through cell surface membrane receptors or indirectly, through the induction of inflammatory cytokines? What control DC migration during *T. gondii* infection. Answers to these questions will go a long way in creating a better understanding of class selection in the T cell immune response to intracellular parasites as well as, provide some general strategies for stimulating host resistance via innate and acquired immunologic components.

ACKNOWLEDGEMENT. This work was supported by grants from the Ministry of Education, Science, Sports and Culture, Japan. The first author was a Foreign Visiting Professor at the National Research Centre for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Japan (2002-2003).

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