Age-related susceptibility and resistance to nonlethal Plasmodium yoelii infection in C57BL/6 mice

Ying Shan1,2, Jun Liu1, Yong-Jun Jiang3,4, Hong Shang3,4, Dong Jiang5 and Ya-Ming Cao1

1Department of Immunology, College of Basic Medical Sciences, China Medical University, No. 92 Beier Road, Heping District, Shenyang 110001, China;
2Department of Immunology and Pathogenic Biology, College of Basic Medical Sciences, Liaoning Medical University, Jinzhou 121000, China;
3Department of Laboratory Medicine, First Hospital of China Medical University, Shenyang 110001, China;
4The Key Laboratory of AIDS Immunology of Ministry of Health, First Hospital of China Medical University, Shenyang 110001, China;
5Department of Anatomy, College of Basic Medical Sciences, Liaoning Medical University, Jinzhou 121000, China

Abstract: In cases of human malaria, children suffer very high rates of morbidity and mortality. To analyse the mechanisms involved in age-dependent protection against malaria, we investigated the characterization of immune responses to Plasmodium yoelii 17XNL (Py 17XNL) in young (3 weeks) and middle-aged (8 months) C57BL/6 mice. In this study, we found that 100% of young mice succumbed to Py 17XNL infection with higher parasitemia, while middle-aged mice were able to clear blood parasites and no mortality was observed. These observations suggested that the young C57BL/6 mice were susceptible to Py 17XNL infection, whereas the middle-aged mice were resistant. Cellular analysis revealed that both the numbers of splenic myeloid dendritic cells (mDCs) as well as the expression of DC maturation markers were higher in middle-aged mice than those in young mice. The numbers of IgG1- or IgG2a-secreting B cells increased markedly in middle-aged mice after infection with Py 17XNL. The dynamic change of the number of CD4+CD25+Foxp3+ regulatory T cells (Tregs) in mice infected with Py 17XNL was also different between the two groups. In addition, the levels of IFN-γ and NO increased in both groups during early parasite infection, while there was also an obvious increase in IL-4 production in the infected middle-aged mice. The change in IL-10 levels following infection was consistent with that of the change in the number of Tregs. The survival of middle-aged mice following Py 17XNL infection was dependent upon the establishment of effective Th1 and Th2 responses and a successful switch between Th1 and Th2 responses, as well as appropriate functioning of Tregs.

Keywords: Plasmodium yoelii 17XNL, age, immune response, dendritic cells, Tregs

Malaria, caused by parasites of the genus Plasmodium Marchiafavae et Celli, 1885, remains one of the leading causes of morbidity and mortality in the world, with a conservative estimate of 350–500 million individuals currently infected and causing in excess of one million deaths per year, mainly in children (Moormann 2009, Pierce and Miller 2009). Moreover, the findings of malaria mortality for the period 1980–2010 showed that the malaria mortality burden is larger than previously estimated (Murray et al. 2012). Events leading to the manifestations of severe malaria in a pediatric population are influenced by multiple intrinsic as well as extrinsic factors. These include, but are not limited to, intensity of malaria transmission, genetic diversity of the infecting parasite and complexity of infection, degree of maternal antibody protection and prenatal malaria experience, fetal hemoglobin S heterozygosity and nutritional status (Moormann 2009). These factors aside, children remain more susceptible to malaria in comparison to adults. This age-associated susceptibility has in part been explained by the fact that children can be immunologically naïve, and repeated infections are required to develop a repertoire of immune cells capable of recognizing the various and antigenic variant malaria-derived proteins. Once infected, however, children are more likely to succumb to the immunopathology associated with malaria (Maitland and Marsh 2004), which is primarily the result of insufficient modulation of TNF-α mediated pro-inflammatory responses induced by parasite molecules and antigens that stimulate innate and adaptive immunity (Clark and Schofield 2000, Arvanitis-Tsakonas et al. 2003). According to human immunoparasitological studies, it appears

Address for correspondence: Y.-M. Cao, Department of Immunology, College of Basic Medical Sciences, China Medical University, No. 92 Beier Road, Heping District, Shenyang, China. Phone: +86 24 23256666 5346; Fax: +86 24 23264417; E-mail: ymcao@mail.cmu.edu.cn
that the underlying pathophysiological determinants in children are still poorly known. Adults living in endemic areas can acquire protection against chronic exposure (Baird 1998, Murphy and Breman 2001). To understand this age-related susceptibility and protection of humans from malaria infection, several studies have demonstrated that the immune response and manifestations to malaria in children and adults are different (Xainli et al. 2002, Perkmann et al. 2005, Ramharter et al. 2005, Vestergaard et al. 2008, Khosav et al. 2011, Lacerda et al. 2012). Comparison of cell-mediated activity of circulating cytokine levels in children and adults in different areas of malaria transmission has shown predominantly Th1-like responses in children, and a tendency to Th2-like responses in adults (Troye-Blomberg et al. 1990, Mshana et al. 1991, Riley et al. 1991, 1992, Elghazali et al. 1995, Luty et al. 1999, Le Hesran et al. 2006). However, it is not clear which immune responses are lacking in infants and young children, yet confer the semi-immune status that benefits adults. Thus, further understanding of the age-dependent characteristics of protection and susceptibility to parasites that cause malaria is necessary for the development of urgently needed vaccines.

The most serious limitation of the progress of characterization of the age-dependent immunological mechanisms in malaria infection was the lack of suitable laboratory animal model. However, over the past several years, the use of resistant, susceptible and genetically deficient mice, together with different Plasmodium strains, has allowed the in-depth dissection of the immunological mechanisms in adult mice (Langhorne et al. 1989, 2002, Taylor-Robinson 1995, Li et al. 2001, Longley et al. 2011). From these studies, it is clear that both cellular and humoral immune responses are required to control and clear malaria parasites.

In one of the previous studies, it was suggested that the phenomenon of age-related susceptibility or resistance to malaria in mice has not been well established (Pierrot et al. 2003). However, that experiment was carried out using the lethal strain, Pb ANKA in mice aged 4, 10 and 16 weeks. Interestingly, it was suggested almost 60 years ago that aged mice (12 months) infected with Pb ANKA survived longer than adult mice (12 weeks) (Greenberg et al. 1953).

In the present study, in order to better understand age-associated differences in immunity to malaria, we used a C57BL/6 mouse model to define differences in cellular, humoral and cytokine responses between young (3 weeks) and middle-aged (8 months) mice infected with Py 17XNL (nonlethal strain), which was described by Landau et al. (1968). We also used this model to investigate whether any of these responses may provide protection against infection in young mice. This mouse model may also be useful to study the phenomenon of age-related susceptibility or resistance to malaria in humans.

**MATERIALS AND METHODS**

**Mice, parasites and experimental infection**

Three week old (young) and 8 month old (middle-aged) female C57BL/6 mice were purchased from Beijing Animal Institute (Beijing, China). Py 17XNL was kindly provided by Dr. Motomi Torii (Department of Molecular Parasitology, Ehime University Graduate School of Medicine, Ehime, Japan). Infections were initiated by intraperitoneal (i.p.) injection of $1 \times 10^5$ Py 17XNL parasitized erythrocytes per mouse in each group of young and middle-aged C57BL/6 mice. Parasitemia was monitored by counting the number of parasite-infected erythrocytes per 1000 erythrocytes by light microscopic examination of Giemsa-stained thin smears of tail blood. All experiments were performed in compliance with local Animal Ethics Committee requirements.

**Spleen cell cultures**

Splenic cell culture was performed as previously described (Chen et al. 2009). Briefly, spleens from normal and infected mice were removed aseptically and pressed through a sterile fine wire mesh with 10 ml RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS), 25mM HEPES, 0.12% gentamicin and 2mM glutamine. Cell suspensions were collected by centrifuging at 350 g for 10 min. Erythrocytes were lysed with cold 0.17M NH4Cl and cells were washed twice with fresh medium. The viability of cells was determined by trypan blue exclusion and was always >90%. Aliquots (500 μl/well) of the cell suspensions ($1 \times 10^7$/ml) were incubated in 24-well flat bottom tissue culture plates (Falcon) in triplicate for 48 h at 37°C in a humidified 5% CO2 incubator. Supernatant fractions were collected and stored at −80°C until they were assayed (Su and Stevenson 2002).

**Flow cytometry analysis**

A portion of the infected mice were sacrificed simultaneously at the indicated times, in order to measure mDCs, the population of CD11c+ DCs expressing maturation markers, MHC II and CD86, Tregs, and IgG1- and IgG2a-secreting B cells. Unless otherwise indicated, antibodies were purchased from BD Biosciences.

Spleen cells from C57BL/6 mice were collected at different time points after infection. To assess DCs, cells were double stained with FITC-conjugated anti-CD11c mAb (clone HL-3) and PE-conjugated anti-CD11b (clone M1/70), anti-CD86 mAb (clone 3/23), and anti-MHC II mAb (clone M5/114.15.2) (Grelli et al. 2004). To assess Tregs, FITC-conjugated anti-CD4 and PE-conjugated anti-CD25 antibodies (clone PC61) were added to spleen cells, and the cells were then resuspended in 100 μl of phosphate-buffered saline supplemented with 3% FCS for surface staining. The cells were then fixed and permeabilized, and intracytoplasmic staining was performed using APC-conjugated anti-Foxp3 antibody (clone FJK16s) (Seixas and Ostler 2005). To measure IgG1- and IgG2a-secreting B cells, the cells were first surfaced-stained with PerCP-conjugated anti-CD45R/B220 (clone RA3-6B2). Cells were then fixed, permeabilized and stained with anti-IgG1 or anti-IgG2a.
The cells were then washed twice with PBS containing 1% FCS and suspended in 300 μl of PBS, and analyzed in FACSCalibur cytofluorometer using CellQuest software. Viable cells were gated by forward and side scattering.

**Detection of cytokines by ELISA**

Levels of IFN-γ, IL-4 and IL-10 were measured by commercial enzyme linked immunosorbent assay (ELISA) kits according to the manufacturer’s protocol (R&D Systems, Minneapolis, MN). The OD values were read in a microplate reader at 450 nm. The concentration of cytokines in each sample was calculated using a standard curve generated using recombinant cytokines.

**Determination of nitrite (NO$_2^-$) concentration**

To determine NO production, concentrations of NO$_2^-$ in cell supernatants were measured by the Griess reaction (Yanez et al. 1996). Briefly, 100 μl of supernatant was incubated with 100 μl of Griess reagent for 10 min at room temperature, and NO$_2^-$ concentration was determined by measuring the optical density at 550 nm (A550) in reference to the A550 of standard NaNO$_2$ solution.

**Statistical analysis**

Data are presented as the means ± standard errors of the means (SE). Statistical significance of the differences was analyzed by t test or one way ANOVA (SPSS 17.0). A value of $p < 0.05$ was considered significant.

**RESULTS**

**Infection course and levels of IFN-γ and NO**

In the present study, young and middle-aged C57BL/6 mice were infected with P.y 17XNL and showed divergent courses of infection and disease severity. Infection with P.y 17XNL was lethal on day 20 to the young mice with peak parasitemia of about 42%. By contrast, middle-aged mice survived infection with P.y 17XNL and developed a moderate parasitemia, which increased steadily from approximately 2% on day 4 post-infection (pi) to 20% on day 12 pi and dropped thereafter (Fig. 1A). These observations indicated that the young C57BL/6 mice were susceptible to P.y 17XNL infection, whereas the middle-aged mice were relatively resistant.

Our previous studies have demonstrated that resistance to blood stage malaria infections depended on the ability to induce an early effective Th1 cell immune response. Therefore, we investigated the level of the Th1 cytokine, IFN-γ in mice following infection with P.y 17XNL. Increased IFN-γ production was observed on day 5 pi in both young and middle-aged mice (Fig. 1B). Similar patterns of NO production were observed in each group during the early stage of infection. As shown in Fig. 1C, NO levels in the culture supernatant of splenocytes from each group peaked on day 5 pi.

![Fig. 1. Parasitemia and levels of IFN-γ and NO in young (3W) and middle-aged (8M) mice infected with P.y 17XNL. Percentages of parasitemia were calculated by counting the number of parasite-infected erythrocytes per 1000 total erythrocytes by light microscopic examination of Giemsa-stained thin smears of tail blood. ELISA was performed to detect levels of IFN-γ in supernatants of cultured spleen cells from infected mice. The concentration of NO$_2^-$ was detected using the Griess reaction. Results were presented as the arithmetic mean of three mice per group ± SE. * $p < 0.05$, ** $p < 0.01$ versus corresponding values for non-infected control mice (0 d); # $p < 0.05$ indicates the comparison between young and middle-aged mice.](image-url)
Number of mDCs and DC maturation markers

Dendritic cells (DCs) are the sole antigen presenting cells (APC) responsible for initiating naïve T lymphocyte activation. Therefore, we examined the myeloid dendritic cells (mDCs) in mice of different ages. We also compared the expression of the DC maturation markers MHC II and CD86 on splenic CD11c+DCs, which are essential for induction of a T cell response. The results revealed that the numbers of mDCs (Fig. 2A), and the expression of MHC II (Fig. 2B) and CD86 (Fig. 2C) increased after infection in each group with a peak on day 8 pi. The peak values were significantly different from those of uninfected mice. Importantly, both the numbers of DCs and the expression of DC maturation markers were significantly lower in young mice than those in middle-aged mice at peak.

IgG1+ or IgG2a+ B cells and IL-4 level

To investigate whether splenic B cells exhibit changes in accordance with the observed age-associated susceptibility and resistance to Py 17XNL, the numbers of IgG1- or IgG2a-secreting B cells were analysed by flow cytometry. The results revealed that the numbers of B cells secreting IgG1 (Fig. 3A) or IgG2a (Fig. 3B) increased obviously in middle-aged mice after infection.
with Py 17XNL, whereas there was no obvious change in the numbers of these B cells in young mice compared to corresponding values for non-infected mice. These results showed that B cells in the middle-aged mice are able to be activated and secrete large amounts of IgG1 and IgG2a when these mice were infected with Py 17XNL.

The activation and secretion of IgG1 or IgG2a by B cells depend on Th2 cytokines, such as IL-4. Therefore, we evaluated the levels of IL-4 in supernatants from cultured spleen cells of infected mice. In middle-aged mice, on day 5 and 8 pi, there was an obvious increase in IL-4 production, which implied a switch in the immune response from Th1 to Th2. There was no obvious change in the levels of IL-4 in young mice compared to the corresponding values for non-infected mice (Fig. 3C).

**Tregs cell number and IL-10 level**

To compare the immunoregulatory effects of Tregs in mice of different ages during malaria parasite infection, flow cytometric analysis was performed. Tregs in splenocytes were evaluated by triple staining with FITC-anti-CD4, PE-anti-CD25 and APC-anti-Foxp3 monoclonal antibodies, in both young and middle-aged mice infected with Py 17XNL. The number of Tregs increased and peaked (absolute cell number, 8.369 × 10^5) on day 3 pi and then decreased in young mice. In adult mice, the number of Tregs increased on day 3 pi and reached a peak (absolute cell number, 1.69 × 10^6) on day 8 pi (Fig. 4A). Importantly, the number of Tregs in middle-aged mice on day 8 pi was much higher than that in young mice.

To demonstrate the potential mechanisms of immunosuppression by Tregs, we evaluated the levels of the Th1 inhibitory cytokine IL-10 in splenocyte culture supernatants from mice infected with malaria parasite. An increase in IL-10 production was found after infection in both groups. The level of IL-10 peaked on day 5 pi in young mice and on day 8 pi in middle-aged mice (Fig. 4B), which was consistent with the change in numbers of Tregs. These results suggested that Tregs had an important role in regulation associated with the induction of IL-10.

**DISCUSSION**

In adults, exposure over several years appears to be necessary for the acquisition of resistance to many parasite variants and for mounting effective immune responses. However, many studies have shown that adults acquire resistance more rapidly than children (Baird et al. 1991, 1993, Baird 1995). To understand this age-related susceptibility and protection of humans to malaria infection, several epidemiology studies have addressed the issue of whether the immune response to malaria in children and adults is different. There may be several mechanisms potentially involved in immune hyporesponsiveness in young mice and humans, such as developmental immaturity of APCs thus influencing establishment of effec-

**Fig. 4.** Number of Tregs and level of IL-10 in young (3W) and middle-aged (8M) mice during Py 17XNL infection. The numbers of CD4^+^CD25^+^Foxp3^+^Tregs in total spleen T cell populations were measured by flow cytometry in C57BL/6 mice after infection with Py 17XNL. ELISA was performed to detect the levels of IL-10 in supernatants of cultured spleen cells from infected mice. Results were presented as the arithmetic mean of three mice per group ± SE. * p < 0.05, ** p < 0.01 versus corresponding values for non-infected control mice (0 d); ## p < 0.01 indicates the comparison between young and middle-aged mice.

tive T cell-APC interactions (Hunt et al. 1994, Marshall-Clarke et al. 2000), incomplete signalling because of low expression of CD40L on T cells (Brugnoni et al. 1994), and/or impaired responsiveness to TLR ligands leading to a lack of appropriate cytokine production (Levy et al. 2004). In experimental malaria, the most extensive work on immune responses has been conducted using adult mouse models with no clear studies on the effects of age. From these studies, it appears that cellular and humoral responses are essential actors in the control and clearance of malaria parasites (Taylor-Robinson 1995, Li et al. 2001, Langhorne et al. 2002). In order to more carefully consider the impact of age on the biology of host-parasite interactions in malaria, we attempted to identify differential changes in the immune response that may occur during infection in young and middle-aged C57BL/6 mice infected with Py 17XNL.

In this study, we found that the course of infection was significantly affected by age, as infection with Py 17XNL resulted in contradictory outcomes in young and middle-aged mice. We showed that young mice (3 weeks) succumbed to Py 17XNL infection, while middle-aged mice (8 months) cleared the parasites and survived the infec-
tion, which indicated that the combination of C57BL/6 mice and Py 17XNL infection may be considered a valuable model for studying the age-related changes of the immune responses during infection.

We previously demonstrated that the uncontrolled proliferation of blood stage malaria parasites was the main cause of death in mice infected with *Plasmodium*. CD4+Th1 immune effectors were found to be crucial in controlling the proliferation of parasites during early infection by triggering cellular immune responses (Urban et al. 2005). However, T lymphocyte activation and differentiation depend upon DCs, which provide a critical role in initiating adaptive immune responses (Banchereau and Steinman 1998). The splenic DC compartment is a heterogeneous population with subsets of cells differing in functions and morphologies (Shortman and Liu 2002). Th1 responses are mainly mediated by mDCs (Liu 2001, Kuwana 2002, Jangpatarapongsa et al. 2008) and thus we compared the numbers of mDCs and DC maturation as measured by the expression of DCs maturation markers, MHC II and CD86, which are essential for induction of T cell responses (Sher et al. 2003, Cervi et al. 2004).

These results showed that both the number of mDCs and the expression of MHC II and CD86 increased significantly after infection on day 8 pi in both young and middle-aged mice, indicating that infection with Py 17XNL could induce the maturation and activation of DCs in both young and middle-aged C57BL/6 mice. The proliferation and activation of DCs allows for the Th1 response to control the proliferation of parasites in early infection in both groups of mice, which was consistent with the previous studies that CD4+ T cells were associated with protection against malaria through the control of growth of blood parasite stages (Meding and Langhorne 1991, Weiss et al. 1993, Phillips et al. 1994).

We also evaluated Th1-type immune responses as measured by the production of IFN-γ and NO. The level of IFN-γ and NO increased markedly in each group following infection. These results demonstrated that successful resistance to blood stage malaria infections in mice depended on their ability to induce an early effective Th1 cellular immune response characterized by predominant IFN-γ secretion, which might promote NO production to control parasite growth as previously described (Jacobs et al. 1995, 1996). The significant difference in NO release between the young and middle-aged mice on day 5 pi could explain that the parasitemia level of middle-aged mice was lower than that of young mice during early infection. Therefore, both young and middle-aged mice infected with Py 17XNL were able to mount effective pro-inflammatory Th1 responses.

IFN-γ production was used as a measure of Th1 responsiveness and IL-4 production was used as a measure of Th2 responsiveness (Achidi et al. 2005). In addition, related studies have shown that effective establishment of a Th1 immune response and a successful switch to a Th2 response are crucial for protective immunity against malaria. Balanced and coordinated Th1/Th2 regulation not only controls the crisis of the acute phase, but also facilitates the ultimate elimination of the malaria-causing parasite (Taylor-Robinson et al. 1993, Malhotra et al. 2005). In this study, we found that there was a successful switch to the Th2 response in the middle-aged mice infected with Py 17XNL. This Th2 response was characterized by a high level of IL-4, and was also supported by the increased numbers of IgG1- or IgG2a-secreting B cells. Secretion of IgG1 and IgG2a antibodies have been shown to be controlled by Th2 cytokines (Binder et al. 1995). It has been demonstrated that specific antibodies are responsible for the clearance of the parasites that cause malaria thereafter (Taylor-Robinson and Phillips 1994, Couper et al. 2005, Yazdani et al. 2006, Moneriz et al. 2011, Clark et al. 2012).

In the current experiments, we demonstrated that Th2 cytokines such as IL-4 promoted B cells to secrete IgG1 and IgG2a, which played a crucial role in the final clearing of parasites from the blood leading to survival of the middle-aged mice after Py 17XNL infection. In the group of young mice, a pro-inflammatory Th1 response was successfully established, but did not successfully switch to an anti-inflammatory Th2 response, thus not producing the specific antibodies that were necessary to clear the parasites.

Immunity against severe malaria may also depend upon the host’s ability to fine tune the magnitude and process of the cellular immune response, which allows appropriate production and timing of inflammatory or anti-inflammatory cytokines at key stages of the infection. A proper balance between pro- and anti-inflammatory immune responses is essential to control the pathogenesis of severe malaria (Walther et al. 2009, Cox-Singh et al. 2011) in which Tregs might serve as an important regulator (Wu et al. 2010, Berretta et al. 2011, Lyke et al. 2012). The suppression of Tregs in malaria infection has been addressed in a rodent model by presenting an increased survival associated with higher T-cell responsiveness against parasite-derived antigens after depletion of Tregs (Hisaeda et al. 2004). Tregs can exert their function in an IL-10 dependent manner (Belkaid et al. 2002, Okamoto et al. 2011). One human study reported a close relationship between increased percentages of Tregs and elevated IL-10 responses to antigen of *P. falciparum* (Welch, 1897) in cord blood mononuclear cells (Brustoski et al. 2006).

In the present study, we showed that the activation of Tregs was accompanied by high levels of IL-10, which was also consistent with our previous studies (Wu et al. 2007, Chen et al. 2009). Here, the number of Tregs peaked on day 8 pi in middle-aged mice infected with Py 17XNL, while in young mice, the number of Tregs peaked on day 3 pi. Moreover, the number of Tregs in middle-aged mice
on day 8 pi was about three-fold higher than in young mice on the same day. Thus, these data showed a marked difference in kinetics of the proliferation of Tregs and the production of the immunoregulatory cytokine IL-10 between the young susceptible and middle-aged resistant mice infected with Py 17XNL. Considering the expansion of Tregs in the middle-aged resistant mice infected with Py 17XNL at the later time point (day 8 pi), we speculated that this proliferation might limit the excessive Th1 reaction and then switch to a Th2 response, resulting in increased antibody production to abrogate the parasites. This speculation is based on the crucial roles of Tregs in regulating Th1/Th2 responses and in the shift of Th1 to Th2 cells (Artavanis-Tsakonas and Riley 2002, Song et al. 2009). In the young susceptible mice, the failure of activation of Tregs at the critical phase for establishing an effective Th2 response (day 8 pi) would lead to an ineffective Th2 response, resulting in the death of these mice. The possible mechanisms remain to be clarified. The previous study also proved that inappropriate functioning of Tregs resulted in the death in young susceptible rats (Adam et al. 2003).

Taken together, the young C57BL/6 mice were susceptible, whereas the middle-aged mice were resistant to Py 17XNL infection, which implies that C57BL/6 mice infected with Py 17XNL may be considered as a model for studying the age-related changes of the immune responses during infection. The middle-aged mice survived after Py 17XNL infection, and survival was dependent on the establishment of effective Th1 and Th2 responses, a successful switch between Th1 and Th2 responses, and appropriate functioning of Tregs. This study also indicated that we could use IL-4 or IL-10 agonist at appropriate time to promote a successful switch between Th1 and Th2 responses in rodent species during the plasmodium infection.

Acknowledgements. We thank Dr. Motomi Torii (Ehime University Graduate School of Medicine, Ehime, Japan) for providing malaria parasite strains of Py 17XNL. We are also grateful to the Department of Immunology, China Medical University, Shenyang for technical help and logistical support during this study.

REFERENCES


CERV I L., MACDONALD A.S., KANE C., DZIERSZINSKI F., PEARCE E.J. 2004: Cutting edge: dendritic cells copulsed with microbial and helminth antigens undergo modified maturation, seg-


