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Down-Regulation of IL-12 p40 Gene in Plasmodium berghei-Infected Mice

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We analyzed the mechanism that causes suppression of IL-12 p40 gene induction during Plasmodium berghei infection. Although IL-12 together with IFN-γ plays an important role in protection against pathogenic infection, the IL-12 p70 protein production of infected macrophages is lower than that by the uninfected macrophages. We showed in the present study that the induction of IL-12 p40 gene but not IL-12 p35 gene in macrophages of P. berghei-infected mice was profoundly inhibited. The inhibition was induced by interaction with macrophages that had contacted with P. berghei-infected erythrocytes and was mediated by a soluble factor, IL-10. There was comparable activation of NF-κB in uninfected and infected cells. The induction of IFN-regulatory factor-1 gene was comparable in transcription level in uninfected and infected cells, while the unidentified complex formation of IFN-regulatory factor-1 was observed in infected cells. Therefore, the inhibition of the IL-12 p40 gene induction appeared to be regulated at transcriptional regulation level of the gene. The Journal of Immunology, 2001, 167: 235–241.

**Materials and Methods**

**Cytokines, Abs, and reagents**

Recombinant murine IL-10 and mAb specific for IL-10 (SXC-1 and SXC-2) were provided by K. Moore (DNAX Research Institute, Palo Alto, CA) (19). Preparation of the Ab specific for T cells was previously described (20). PGE2 and indomethacin were purchased from Sigma (St. Louis, MO). Recombinant IFN-regulatory factor-1 (IRF-1) was prepared by transforming Escherichia coli with pET expression plasmid containing IRF-1 cDNA that was provided by M. Sato (University of Tokyo, Tokyo, Japan).

**Mice**

Eight- to 10-wk-old C57BL/6 mice were purchased from Charles River Japan (Yokohama, Japan). All mice were used in accordance with our institutional guidelines for animal experimentation.

**Experimental infections and pathogens**

P. berghei (ANKA strain) protozoa were provided by S. Kojima (University of Tokyo) and were maintained in our animal facility by blood passages. Mice injected with thioglycolate (TGC) 3 days earlier were infected i.p. with $1 \times 10^7$ parasitized erythrocytes, and PECs were prepared from the mice on day 1.

**Isolation and stimulation of PECs for cytokine gene induction**

PECs taken from mice treated with TGC 4 days earlier were allowed to adhere on tissue culture dishes for 2 h, and nonadherent cells were removed. PECs were cultured in the presence of LPS (10 μg/ml) plus recombinant mouse IFN-γ (100 U/ml; Genzyme, Cambridge, MA). Total RNA was extracted 2–16 h later and subjected to RT-PCR for IL-12 p40.
IL-12 p35, TNF-α, IL-10, inducible NO synthase (iNOS), IRF-1, TGFβ1, TGFβ2, eotaxin, IFN-inducible protein-10, RANTES, monocytic chemotactic protein-1, monokine induced by IFN-γ, and macrophage inflammatory protein-1β. The medium used was RPMI 1640 supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM sodium pyruvate, 1 time nonessential amino acid, 50 μM 2-ME, and 10% heat-inactivated FCS.

Cytokine ELISA

PECs were stimulated with LPS (10 μg/ml) plus recombinant mouse IFN-γ (100 U/ml; Genzyme) in vitro for 16 h. The amounts of IL-12 p70 in the culture supernatant were determined by sandwich ELISA established with mAbs that were purchased from BD PharMingen (San Diego, CA). Recombinant mouse IL-12 p70 proteins were provided by Genetics Institute (Cambridge, MA) and were used as a standard.

RNA isolation and mRNA detection by RT-PCR

Total cellular RNA was isolated by the guanidinium-thiocyanate method. Equal amounts of RNA (1 μg) were reverse transcribed using 200 U of reverse transcriptase (Moloney murine leukemia virus; Promega, Madison, WI), 1 mM of dNTP, and 100 ng of random primers (Takara Shuzo, Kyoto, Japan) in a total volume of 20 μl. Reverse transcription was conducted at 42°C for 15 min and 30 μl of RNase-free Tris-EDTA buffer was added to each sample. PCR amplification was conducted in a GeneAmp PCR System 9600 (Perkin-Elmer, Norwalk, CT) using 1 ml of the reverse-transcribed product and 0.5 μl of PCR polymerase (Takara Shuzo) in a final volume of 20 μl. The reaction conditions were as follows: DNA denatured at 94°C for 5 min, predetermined cycles at 94°C for 30 s, at the predetermined annealing temperature for 30 s and 72°C for 1 min, and DNA extension at 72°C for 10 min. Primers and cycles used were summarized in Table I. The PCR products were visualized and recorded with Fujifilm Digital Image File DF-20 and the Sony CCD Video Camera Module XC-75/75CE (Tokyo, Japan), and the intensities of the bands were determined by a computer (Adobe Photoshop 2.5J (Adobe Systems, Mountain View, CA) and NIH Image software (National Institutes of Health, Bethesda, MD)).

To measure the RT-PCR product semi-quantitatively, cDNA or RT-PCR products were inserted into pBluescript or pBlueT plasmid (Stratagene, La Jolla, CA), respectively, and were used as standard calculating curves. We drew a standard curve for a gene of interest to directly compare the amount of the PCR products. A graded dose of a gene fragment and β-actin DNA fragment were used as templates, and the PCR products were visualized in agarose gel containing ethidium bromide and recorded. The intensities of the bands were measured by NIH Image software. The results were plotted on semilog graph paper, the intensity (y-axis) on a linear scale, and the amount of template (x-axis) on a log scale. Both β-actin and IL-12 p40 gave a straight line with an r² of 0.997 and 0.999, respectively. The level of PCR products of a gene were expressed as femtograms per picogram or nanograms per microgram. The relative abundance of PCR products of a gene of interest to directly compare the amount of PCR products of different genes were expressed as femtograms per picogram or nanograms per microgram. The results were plotted on semilog graph paper, the intensity (y-axis) on a linear scale, and the amount of template (x-axis) on a log scale. The intensity of the bands was determined by NIH Image software (National Institutes of Health, Bethesda, MD))

Northern blot analysis

The procedure for RNA blot analysis has been described in Harada et al. (21). Fragments of IL-12 p40, IL-12 p35, and TNF-α were labeled by the PCR method to prepare DNA probes.

EMSA

EMSA analysis was done as described by Harada et al. (21). Nuclear extract of PECs was incubated with a 32P-labeled DNA probe in a buffer containing 10 mM Tris-Cl (pH7.5), 50 mM NaCl, 1 mM DTT, 1 mM EDTA, and 5% glycerol at room temperature for 30 min. Protein-DNA complexes were analyzed by 4% PAGE. Probes used for detecting NF-κB and IRF-1 were NF-κB site (5’-GAT CTA GGA GAC TTT CCC TAG C-3’) and IRF element (5’-AGA GGA AAA CTG AAA GGG AGA ACT-3’), respectively (22, 23).

Flow cytometry

Monoclonal Abs used for staining were fluorescein-labeled anti-CD11b, anti-CD11c, anti-CD40, anti-CD80, and anti-CD86. These mAbs were purchased from BD PharMingen. Cells were incubated with the indicated Ab in the presence of anti-FcR Ab (2.4G2). Stained cells were analyzed on a FACSCalibur with the CellQuest software (BD Biosciences, Mountain View, CA).

Statistical analysis

Student’s t test was used for testing the significance of the difference between the experimental groups.

Results

IL-12 p70 production is inhibited in PECs of P. berghei-infected mice

To determine whether P. berghei infection influences macrophage production of IL-12 protein, mice were infected with P. berghei blood-stage parasites, and TGC-induced PECs were collected on day 1. The PECs were stimulated in vitro with LPS plus IFN-γ for 16 h, and the level of IL-12 p70 protein in the culture supernatant was measured by ELISA (Fig. 1). IL-12 p70 was not detected in the unstimulated culture supernatant of PECs. The amount of IL-12 p70 gradually increased and reached a plateau at 8 h post-stimulation in uninfected PECs and control RBC-treated PECs, while the level was lower at all points in time in P. berghei-infected PECs. This result indicates that the observed inhibition of IL-12 production is not due to the nonspecific effect of injecting RBC nor contaminant in TGC. Rather the result indicates that the inhibition is specific for injecting P. berghei-infected RBC. Therefore, only TGC-injected mice were used as a control in the further study described in this report.

Table I. Primers used for PCR

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<th>Genes</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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<th>Cycles</th>
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<td>CGGTTACCTCACCACATGTCTACG</td>
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<tr>
<td>IL-12 p35</td>
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Expression of IL-12 p40 gene and IL-12 p35 gene in PEC is differentially regulated

Cytokine gene expressions in the PECs of uninfected and P. berghei-infected mice are compared in Fig. 2. IL-12 p40 gene induction was strongly suppressed in PECs of P. berghei-infected mice at all points in time that were tested, while IL-12 p35 gene induction in PECs of infected mice was rather enhanced. TNF-α were induced in uninfected and infected mice at comparable levels (Fig. 2A). The result was also confirmed by Northern blot analysis (Fig. 2B). Therefore, the expression of IL-12 p40 and p35 gene in P. berghei-infected mice is differentially regulated, and P. berghei infection specifically inhibits the IL-12 p40 gene expression.

The PECs are CD11b positive

Cell surface markers of PECs were analyzed with flow cytometry. PECs were recovered from plastic dishes by treatment with EDTA and were stained with the indicated FITC-conjugated Ab. The representative results were shown in Fig. 3. The PECs were highly positive for CD11b and slightly positive for CD11c. This indicates that the majority of the PECs are macrophages. They also expressed CD40, CD80, and CD86. The level of the expression was the same between uninfected and P. berghei-infected PECs except CD80. The expression level of CD80 on P. berghei-infected PECs was higher than that of uninfected PECs.

Comparable expression of chemokine gene expression in uninfected and P. berghei-infected PECs

The induction of the chemokine gene was analyzed in uninfected and P. berghei-infected PECs by RT-PCR. There was no significant difference in expression of the genes between the groups (data not shown).

P. berghei-infected erythrocytes were phagocytized by PECs

To learn whether PECs phagocytize P. berghei-infected erythrocytes, PECs were prepared 1 day after the injection of P. berghei-infected erythrocytes, stained with Giemsa, and observed under photomicroscopy. Binding of erythrocytes of P. berghei-infected mouse origin to the PECs and uptake of erythrocytes of P. berghei-infected mice by the PECs (11.2 and 5.1%, respectively) were frequently observed. A small proportion (<1%) of PECs carried the parasitized erythrocytes in the cytoplasm (data not shown). Binding or uptake of erythrocytes of uninfected mouse origin were barely observed.
Inhibition of IL-12 p40 gene induction in P. berghei-infected PECs

Although *Plasmodium* parasites do not directly infect to PECs, the reactivity of the cells is profoundly influenced by *Plasmodium* infection. The event occurred in vivo within a day after infection with *Plasmodium* parasites. PECs of uninfected and *P. berghei*-infected mice were cocultured in vitro and were stimulated with LPS plus IFN-γ to determine what kind of cells were responsible for the observed inhibition (Fig. 4A). RNA extracted from the 1:1 mixed-culture of uninfected and infected PECs exhibited a less than average value for the IL-12 p40 mRNA expression of the uninfected and infected groups. TNF-α mRNA induction was comparable in these groups. The result demonstrated the presence of active intercellular inhibition of the gene induction during *P. berghei* infection. The inhibition is not mediated by T cells that might have contaminated the PECs, because PECs cytotoxically treated with anti-T cell Ab were inhibited (Fig. 4B).

Further testing was conducted to determine whether direct cell-to-cell interaction is required for the inhibition observed above. The culture supernatant of unstimulated PECs of uninfected and *P. berghei*-infected mice was tested for its effect on IL-12 p40 gene induction by PECs of uninfected mice (Fig. 4C). It was shown that the addition of the culture supernatant of *P. berghei*-infected PECs inhibited the induction of IL-12 p40 of uninfected PECs. This result suggests that the inhibition of the IL-12 p40 gene observed in the present study is mediated by a soluble factor.

**FIGURE 4.** Intercellular inhibition of IL-12 p40 gene induction in *P. berghei*-infected PECs. A. PECs of uninfected and *P. berghei*-infected mice were mixed (2.5 × 10^6 to 2.5 × 10^6 mixture and 4.5 × 10^6 to 0.5 × 10^6 mixture) and were cultured on tissue culture plates for 24 h. Adherent cells were further cultured in the presence of 10 μg/ml LPS plus 100 U/ml IFN-γ. To measure the mRNA expression of TNF-α and IL-12 p40, RNA was extracted at 2 h to measure the mRNA expression of TNF-α and at 8 h to measure the mRNA expression of IL-12 p40. Similar results were obtained in four independent experiments. B. PECs of uninfected and *P. berghei*-infected mice were mixed (2.5 × 10^6 to 2.5 × 10^6 mixture) and were cultured on tissue culture plates for 24 h. PECs of infected mice were treated with or without anti-T cell Ab and complement. Similar results were obtained in four independent experiments. C. A total of 5 × 10^6 PECs of uninfected and *P. berghei*-infected mice were cultured without stimulation for 16 h, and the culture supernatant was collected and filtrated through 0.22-mm filters. The culture supernatant was added at a final concentration of 5% to 5 × 10^6 of PECs from uninfected mice, and the PECs were stimulated with 10 μg/ml LPS plus 100 U/ml IFN-γ. RNA from the cells was prepared 8 h later and subjected to RT-PCR analysis. Similar results were obtained in two independent experiments.

Inhibition is due to the active inhibition by IL-10 produced by infected cells

It has been reported that IL-12 p40 gene expression is suppressed by PGE_2, IL-10, and TGFβ (7, 8, 24–26). It is also been demonstrated that *Plasmodium* parasites produce PGE_2 (27). Therefore, the observed inhibition may be due to the production of PGE_2, IL-10, or TGFβ by the infected cells or parasites.

We first tested the possibility of involving PGE_2 by adding PGE_2 to PECs in vitro or by treating mice with indomethacin in vivo. Pretreatment of PECs from uninfected mice with PGE_2 inhibited the induction of IL-12 p40 gene by LPS plus IFN-γ to some extent. However, pretreatment of PECs from *P. berghei*-infected mice with indomethacin did not revert the inhibition of the gene induction (data not shown). These treatments revealed a minimal to moderate effect on TNF-α gene induction. Cox-2 gene induction was inhibited by pretreatment of PECs with indomethacin, indicating conditions were sufficient to block the PGE_2 system of the PECs (data not shown). These results demonstrate that the inhibition of IL-12 p40 gene induction observed in *P. berghei*-infected PECs is not due to the effects of PGE_2.

We next determined the expression of mRNA of IL-10, TGFβ1, and TGFβ2. The expression of TGFβ1 and TGFβ2 was inhibited in the ex vivo state. Induction of TGFβ2 but not TGFβ1 mRNA was strongly suppressed in *P. berghei*-infected PECs (Fig. 5). Involvement of TGFβ in the inhibition of IL-12 production was unlikely in the present study, because TGFβ1 was induced at comparable levels in uninfected and infected cells. In addition, the expression of TGFβ2 was one-tenth that of TGFβ1 and was strongly inhibited in infected cells.

In contrast, *P. berghei*-infected augmented the induction of IL-10 by stimulation with LPS plus IFN-γ (Fig. 5). To learn whether IL-10 is responsible for the inhibition of IL-12 production observed in *P. berghei*-infected PECs, we set up the following experiments. PECs of uninfected and *P. berghei*-infected mice origin were cultured with or without stimulation with LPS plus IFN-γ, and the amount of IL-10 in the culture supernatant was determined (Fig. 6A). PECs of *P. berghei*-infected mice produced the IL-10 even in the absence of stimulation. To determine whether this production of IL-10 by PECs of *P. berghei*-infected mice is responsible for the inhibition of IL-12 p70 production, PECs were stimulated in vitro with LPS plus IFN-γ in the presence or absence of anti-IL-10 mAb, and the amount of IL-12 p70 protein in the culture supernatant was measured. As shown in Fig. 6B, the addition of anti-IL-10 mAb reverted the inhibition of IL-12 p70 production and IL-12 p40 mRNA transcription by *P. berghei*-infected...
PECs in a dose-dependent manner. It was also shown that the production of IL-12 p70 by uninfected PECs was augmented by the addition of anti-IL-10 mAb. These results demonstrate that the inhibition of IL-12 p70 production observed in P. berghei-infected PECs is due to the effect of IL-10. This was further confirmed by the addition of rIL-10 to the stimulation culture of PECs (Fig. 6C). The addition of IL-12 p70 in the culture supernatant was measured by ELISA after 24 h of stimulation. Similar patterns were observed in three independent experiments. P. berghei-infected PECs of uninfected and infected mice. Anti-IL-10 Ab was also added in the indicated group. Similar patterns were observed in three independent experiments.

IRF-1 and NF-κB are functioning at comparable level in uninfected and infected PECs

We showed that the induction of IL-12 p40 gene by LPS plus IFN-γ requires transcription factors IRF-1 and NF-κB (18, 28). To evaluate whether those two nuclear factors are functioning in PECs of uninfected and infected mice, iNOS gene expression, which also required NF-κB and IRF-1 (29, 30), was first tested by RT-PCR (Fig. 7A). The gene induction of IL-12 p40 was suppressed in PECs of infected mice. Using the same reverse transcription product, we tested the expression of iNOS and IRF-1 mRNA. iNOS mRNA was expressed at comparable levels in uninfected and infected PECs. IRF-1 mRNA expression was elevated at comparable levels in both groups of PECs by the induction with LPS plus IFN-γ. These results suggest that the observed inhibition of IL-12 p40 gene induction in infected PECs was not due to the low induction of NF-κB or IRF-1.

The binding function of NF-κB and IRF-1 at the protein level was confirmed by EMSA (Fig. 7B). Nuclear extracts were prepared from uninfected and P. berghei-infected PECs that had been stimulated in vitro for the indicated period with LPS plus IFN-γ. Both extracts exhibited binding activity to the probe for NF-κB, indicating that the stimulation with LPS plus IFN-γ induced the activation of NF-κB equally in uninfected and infected PECs. However, the patterns of the IRF-1 binding were different between infected and uninfected groups. Small and large molecules bound to the probe for IRF-1, and both bands disappeared by treatment with anti-IRF-1 Ab (data not shown). The larger size band appeared in the uninfected group 4 h after stimulation. In contrast, the larger size band was detected in the infected group even without stimulation. The intensity of smaller size band was rather stronger in infected group than that in uninfected group. These results suggest that the IRF-1 might form complex with other molecules and the complex formation was accelerated in P. berghei-infected mice.

Discussion

In the present study, we showed that the induction of the IL-12 p40 gene but not the IL-12 p35 gene was strongly inhibited in P. berghei-infected PECs. The inhibition was actively induced by

PECs of uninfected and P. berghei-infected mice were cultured without stimulation or were stimulated with 10 μg/ml LPS plus 100 U/ml IFN-γ in the presence or absence of the indicated amount of anti-IL-10 mAb. The expression of mRNA for IL-12 p40 was determined after 8 h of stimulation. The amount of IL-12 p70 in the culture supernatant was measured by ELISA after 24 h of stimulation. Similar patterns were observed in two independent experiments. P. berghei-infected mice were cultured with 10 μg/ml LPS plus 100 U/ml IFN-γ for 24 h in the presence or absence of 0.5 ng/ml rIL-10. The expression of mRNA for IL-12 p40 was determined after 8 h of stimulation. The amount of IL-12 p70 in the culture supernatant was measured by ELISA after 24 h of stimulation. Similar patterns were observed in three independent experiments. P. berghei-infected mice. IRF-1 mRNA expression was elevated at comparable level in uninfected and infected PECs. IRF-1 mRNA expression was elevated at comparable levels in both groups of PECs by the induction with LPS plus IFN-γ. These results suggest that the observed inhibition of IL-12 p40 gene induction in infected PECs was not due to the low induction of NF-κB or IRF-1.

Effects of in vitro treatment with anti-IL-10 mAb on IL-12 p70 production. A, PECs of uninfected and P. berghei-infected mice were cultured without stimulation or were stimulated with 10 μg/ml LPS plus 100 U/ml IFN-γ for 24 h. The amount of IL-10 in the culture supernatant was measured by ELISA. B, PECs of uninfected and P. berghei-infected mice were stimulated with 10 μg/ml LPS plus 100 U/ml IFN-γ in the presence or absence of the indicated amount of anti-IL-10 mAb for 24 h. The expression of mRNA for IL-12 p40 was determined after 8 h of stimulation. The amount of IL-12 p70 in the culture supernatant was measured by ELISA after 24 h of stimulation. Similar patterns were observed in three independent experiments. C, PECs of uninfected and P. berghei-infected mice were cultured without stimulation or were stimulated with 10 μg/ml LPS plus 100 U/ml IFN-γ for 24 h in the presence of culture supernatant of PECs of uninfected or P. berghei-infected mice. Anti-IL-10 Ab was also added in the indicated group. Similar patterns were observed in three independent experiments.

In Fig. 4C, it was shown that the culture supernatant of P. berghei-infected PECs inhibited the induction of IL-12 p40 of uninfected PECs. Therefore, we asked whether the inhibitory activity of the P. berghei-infected PECs was mediated by IL-10. As shown in Fig. 6D, the inhibitory activity was neutralized by the addition of anti-IL-10 Ab.

IRF-1 and NF-κB are functioning at comparable level in uninfected and infected PECs.

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FIGURE 7. Comparable induction of IRF-1 and NF-κB in uninfected and infected PECs. A. PECs of uninfected (○) and P. berghei-infected (●) mice were stimulated in vitro with 10 μg/ml LPS plus 100 U/ml IFN-γ. mRNA expression of IL-12 p40, iNOS, and IRF-1 gene was measured by RT-PCR at the indicated points in time after stimulation. In this particular experiment, the reverse transcription products of Fig. 3 were used. Similar patterns were observed in three independent experiments. B. Nuclear extracts were prepared at the indicated points in time from uninfected and P. berghei-infected PECs after stimulation with LPS plus IFN-γ. Two micro-liters of nuclear extract were incubated with a 32P-labeled DNA probe of NF-κB or IRF-1 binding site in a buffer containing 10 mM Tris-HCl (pH 7.6), 50 mM NaCl, 1 mM DTT, 1 mM EDTA, and 5% glycerol at 25°C for 60 min.

IL-10 produced by PECs from P. berghei-infected mice. The inhibition was not at the level of activation of NF-κB or IRF-1 transcription factors. Rather, it is suggested that the inhibition might be at the transcriptional regulation level.

Microorganisms activate macrophages to produce IL-12. IL-12 subsequently stimulates NK cells to produce IFN-γ, which in turn activates macrophages to stimulate Ag-specific T cells for eradication of microbial pathogens. In this type of immune response, IL-12 together with IFN-γ plays a central role for protection against pathogenic infection. However, the present study demonstrates that the inducibility of IL-12 p40 gene was inhibited even in the early phase of P. berghei infection. Although a similar suppression phenomenon in IL-12 gene expression has been reported during measles and Leishmania major infection (15, 16), the mechanism of suppression has yet to be elucidated. In the present study, it was shown that the PECs of P. berghei-infected mice actively inhibit the gene induction of IL-12 p40 but not IL-12 p35 of uninfected PECs. These results suggest that the gene expression of IL-12 p40 and IL-12 p35 is differentially regulated in PECs of P. berghei-infected mice.

P. berghei blood-stage parasites grow in erythrocytes and reenter using specific receptors on erythrocytes, and these parasites do not penetrate macrophages (31). However, the above results show that infection with blood-stage parasites influences the gene activation in macrophages. Recently, it was reported that the Plasmodium-infected erythrocytes bind to dendritic cells and inhibit the function of the dendritic cells (32, 33). In the present study, soluble factor-mediated interaction between uninfected and infected macrophages was shown to be responsible for the inhibition of IL-12 p40 gene. It was observed that the substantial proportion of macrophages were found to bind to and ingested the erythrocytes of the P. berghei-infected mice origin. The culture supernatant of P. berghei-infected PECs induced the inhibition of IL-12 p40 gene induction in uninfected PECs. It has been reported that the Plasmodium-infected erythrocytes inhibit the function of dendritic cells by attaching to the cells (17). This information all taken together suggests that macrophages engulf erythrocytes of the P. berghei-infected mice origin subsequently inhibit the other PECs via a soluble factor.

What is the molecule that mediates the inhibition of IL-12 p40 gene expression observed in P. berghei-infected mice? It was recently reported that IL-12 production in TGC-elicited PECs could be suppressed by TNF-α (34–36). In the present study, induction of TNF-α gene was comparable in PECs of uninfected and P. berghei-infected mice. In addition, exogenous TNF-α did not inhibit the induction of IL-12 p40 mRNA in the present experimental condition (data not shown). Therefore, TNF-α is not responsible for the inhibition of the IL-12 p40 gene expression in P. berghei-infected PECs. Controversial reports have been published on the suppressive effect of TGFβ on IL-12 gene expression (37–39). However, involvement of TGFβ was unlikely in the present study, because TGFβ1 was induced at comparable levels in uninfected and infected cells. In addition, the expression of TGFβ2 was one-tenth that of TGFβ1 and was strongly inhibited in infected cells. Therefore, TGFβ did not appear to be responsible for the inhibition of IL-12 p40 gene expression in infected cells.

IL-10 and PGE2 have also been known to suppress IL-12 gene induction (7, 8, 24, 25, 39). Although the addition of PGE2, to the induction culture of PECs inhibited the IL-12 p40 gene expression of uninfected PECs, the in vivo administration of indomethacin, or in vitro treatment of P. berghei-infected PECs with indomethacin, was not capable of neutralizing the inhibition (data not shown), indicating that PGE2 is not responsible for the inhibition. In contrast, the addition of anti-IL-10 mAb was capable of neutralizing the inhibition. Therefore, IL-10 appears to be the effector molecule that mediates the inhibition of IL-12 p40 gene induction in P. berghei-infected PECs. Although the addition of anti-IL-10 mAb does not fully neutralize the inhibition observed in P. berghei-infected PECs (Fig. 6B), anti-IL-10 neutralized completely the inhibitory activity of culture supernatant of P. berghei-infected PECs (Fig. 6D). This indicated the possibility that the reactivity of the infected PECs is distinct from that of uninfected PECs. This suppression may result in the impairment of IFN-γ production in the infected mice and may reduce the development of a protective cell-mediated immunity in vivo via a NO-dependent mechanism (40, 41).

We previously showed that the induction of IL-12 p40 gene requires IRF-1 and NF-κB (18, 28). These two transcription factors are also required for the induction of iNOS gene (29, 30, 42, 43). If the inhibition of the IL-12 p40 gene observed in the present study is due to the low induction of these transcription factors, IRF-1 and NF-κB, the induction of iNOS gene might be also affected by infection. However, that was not the case. The iNOS gene was induced at comparable levels in infected and infected PECs after stimulation. NF-κB was equally activated between uninfected and infected PECs at the mRNA and protein level. In addition, there was no significant difference in the level of IRF-1 gene induction between uninfected and infected PECs at the mRNA level. However, IRF-1 obtained from infected PECs appeared to form a complex with other proteins in the nuclear extract (upper band in Fig. 7B) even at the 0-h time point. The complex formation might be a regulatory mechanism of IL-12 p40 gene transcription, because similar complex formation was also found in...
uninfected PECs at the 4-h time point. It was reported that the IRF-1 interacts with IFN consensus sequence binding protein (44). However, the Ab against IFN consensus sequence binding protein did not interfere with the binding of the complex to the IRF-1 binding sequence in the present study (data not shown). Taking all these results together, the low inducibility of the IL-12 p40 gene in infected PECs was not due to the low induction of NF-κB or IRF-1. These results instead suggest the possibility that the mechanism of the inhibition might be present at the transcriptional regulation level of the gene.

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References