ACTA MED OKAYAMA 2000; 54(5): 201-209

Calcineurin Antagonists Inhibit Interferon-gamma Production by Downregulation of Interleukin-18 in Human Mixed Lymphocyte Reactions

Masahiko KUINOSE, Hiromi IWAGAKI*, Yoshinori MORIMOTO, Hideo KOHKA, Kenta KOBASHI, Hiroshi SADAMORI, Masaru INAGAKI, Naoto URUSHIHARA, Takahito YAGI and Noriaki TANAKA[a]

Department of Surgery I, Okayama University Medical School, Okayama 700-8558, Japan

Tacrolimus (FK-506) and cyclosporin A (CsA) are calcineurin antagonists used widely as T-cell immunosuppressants; however, their relative efficacy on the production of interleukin-18 (IL-18) remains undefined. We have examined the effects of FK-506 and CsA on the cytokine generation of human peripheral blood mononuclear cells (PBMCs) in mixed lymphocyte reaction (MLR) with lipopolysaccharide (LPS). We studied the levels of interleukin-18 (IL-18), IL-12, IL-10, IL-6, IL-2 and interferon-γ (IFN-γ) in the supernatant in allo-MLR by ELISA assay. Supernatant levels of IFN-γ, IL-2, IL-6, IL-10 and IL-12 were detected 12 h after MLR and markedly increased thereafter. In contrast, production of IL-18 was detected at 12 h, reached a near maximum level at 24 h and decreased at 72 h. These results suggested that IFN-γ production depended on IL-18, IL-12 and IL-2 in the early phase of MLR and depended mainly on IL-12 and IL-2 in the late phase. Both calcineurin antagonists inhibit the generation of IL-18, which plays a large role in allogeneic cell interactions, in macrophages and they also promote an equivalent down-regulation of T helper1(Th1) and Th2 responses in a concentration-dependent manner. About 90% of IFN-γ production induced by MLR was inhibited by an anti-IL-18 antibody, showing that IL-18 can trigger IFN-γ production in MLR. These results suggest that dual signaling consisting of antigen-driven nuclear factor of activated T cells (NFAT) activation and LPS-mediated NF-κB activation is crucial for IL-18 production in macrophages, and that IL-18 can trigger IFN-γ production in T-cells by MLR.

Key words: tacrolimus, cyclosporin, calcineurin antagonist

Interleukin-18 (IL-18) was identified as a cytokine that induced interferon-γ (IFN-γ) in the sera of mice with septic shock (1). IL-18 is secreted from lipopolysaccharide (LPS)-activated macrophages but also from a wide variety of cells. Subsequent studies have shown that IL-18 performs a wide variety of activities in various cells, and recent reviews summarize these activities (2, 3). The cytokine was found to enhance production of T helper 1 (Th1)-type cytokines (IFN-γ and granulocyte/macrophage-colony stimulating factor (GM-CSF)) but not Th2 cytokines (IL-4 and IL-10) in nonadherent murine spleen cells and human peripheral blood mononuclear cells (PBMCs) with T cell receptor (TCR)/CD3 stimulation (1, 4, 5). It was also reported that IL-18 induced the production of IL-2 and IFN-γ and proliferation of TCR/CD3-stimulated murine Th1 clones; however, the cytokine did not affect the production of IL-4 nor the proliferation of Th2 clones *in vitro* (6, 7). In a previous study, we demonstrated that IL-18 upregulated ICAM-1 expression in a KG-1 monocytic cell line through an IFN-γ-independent pathway (8) and that IL-18 is involved in allo-mixed lymphocyte reactions (MLR) (9).

Tacrolimus (FK-506) and cyclosporin (CsA) are calcineurin antagonists used widely as T-cell immunosuppressants to prevent allograft rejection after solid organ transplantation. Investigations have indicated that uses for these agents also include prevention of graft-versus-host disease after allogeneic bone marrow transplantation and treatment of severe, steroid-dependent asthma. FK-506 and CsA act through ligation of distinct intracellular binding protein targets (FK binding protein for FK-506,

* To whom correspondence should be addressed.

cyclophilin A for CsA). These drug-binding protein complexes inhibit the function of calcineurin, which normally activates various transcription factors, including nuclear factor of activated T cells (NFAT) and nuclear factor-κB (NF-κB), that regulate the expression of key cytokines and signaling proteins (9, 10). The potential differential regulation of human antigen-stimulated T-cell subsets by calcineurin antagonists has not been fully described. We herein present a comprehensive study of the effects of calcineurin antagonists on cytokine generation, including that of IL-18, in an allo-MLR system.

Materials and Methods

Reagents. One mM each of FK-506 and CsA were dissolved in ethanol and further diluted in medium and added to the cultures. FK-506 and CsA were generous gifts from Fujisawa Pharmaceutical Co., Ltd. (Ibaraki, Japan) and Sandoz Ltd (Basel, Switzerland), respectively. Anti-recombinant human (rHu) IL-18 antibody (Ab) and anti-HuIFN-γ Ab were prepared as described elsewhere (4). Anti-HuIL-12 Ab and anti-HuIL-2 Ab were purchased from PharMingen (San Diego, CA, USA).

Culture conditions in mixed lymphocyte reaction (MLR). Human peripheral blood mononuclear cells (PBMC) were isolated from buffy coats of 5 healthy volunteers by centrifugation on a density gradient of Ficoll-Paque (Pharmacia Uppsala, Sweden). The PBMC were then washed 3 times in an RPMI 1640 medium (Nissui Co., Tokyo, Japan) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 20 μg/ml kanamycin, and 100 μg/ml streptomycin and penicillin (Sigma Chemical Co., St. Louis, MO, USA). The PBMC were suspended at a final concentration of 1.25×10^5 cells/ml in the RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated FBS. In the MLR, cells from an individual were mixed with cells from an unrelated person. The final concentration of cells was adjusted to 2.5×10^5 cells/ml. Triplicate wells were incubated with 5 μg/ml LPS (Sigma). The plates were incubated at 37 °C in 5% CO_2/95% air for 72 h, after which the supernatants were aspirated and stored at -20 °C until being assayed for cytokines. When the effect of calcineurin antagonists or other reagents was to be examined, all reagents were added to the media at the start of incubation and cultured for 48 h under the same condition. All experiments were performed in at least triplicate samples.

Cytokine assays. The cytokines were measured using ELISAs employing the multiple Abs sandwich principle (for IL-18, MBL; for other cytokines, Quantikine, R & D systems, Minneapolis, MN, USA). The detection limits of the ELISAs for IL-18, IL-12, IL-10, IL-6 and IFN-γ were 10 pg/ml, and for IL-2 it was 5 pg/ml.

Statistical analysis. Data are expressed as mean $\pm$ SEM. For mean comparisons, one-way analysis of variance (ANOVA) was used. Differences were considered statistically valid at $P < 0.05$.

Results

(1) Kinetics of IL-18 production in MLR

Il-18 production was observed 12 h after MLR, reached a near maximum level at 24 h, and decreased at 72 h (Fig. 1).

(2) Kinetics of Th1 cytokine productions in MLR

Production of IL-12, IL-2 and IFN-γ was upregulated at 12 h and increased during the culture period. (Fig. 2).

(3) Kinetics of Th2 cytokine productions in MLR

Production of IL-6 and IL-10 was upregulated at 12 h and reached a near maximum level at 24 h (Fig. 3).

(4) Effect of FK-506 and CsA on IL-18 production in MLR

Both calcineurin antagonists induced significant concentration-dependent inhibition of IL-18 production in MLR. The concentration of FK-506 required for complete inhibition was approximately 10 times lower than that of CsA (Fig. 4).

(5) Effect of FK-506 and CsA on Th1 cytokine productions in MLR

Both calcineurin antagonists induced significant concentration-dependent inhibition of Th1 cytokines produced in MLR. The concentration of FK-506 required for complete inhibition was approximately 10 times lower than that of CsA (Fig. 5).

(6) Effect of FK-506 and CsA on Th2 cytokine productions in MLR

Both calcineurin antagonists induced significant concentration-dependent inhibition of Th2 cytokines produced in MLR. The concentration of FK-506 required for complete inhibition was approximately 10 times lower

than that of CsA (Fig. 6).

(7) Blocking test in MLR

To investigate cytokine interactions in MLR, we examined the effect of anti-IL-18 Ab, anti-IL-12 Ab, anti-IL-2 Ab and anti-IFN-γ Ab on production of IFN-γ and IL-10 at 48 h. The addition of these Abs significantly reduced IFN-γ production in MLR. In contrast, amounts of IL-10 were significantly downregulated after the addition of anti-IL-18 Ab and anti-IFN-γ Ab, but they were not downregulated by the addition of anti-IL-12 Ab and anti-IL-2 Ab (Fig. 7).

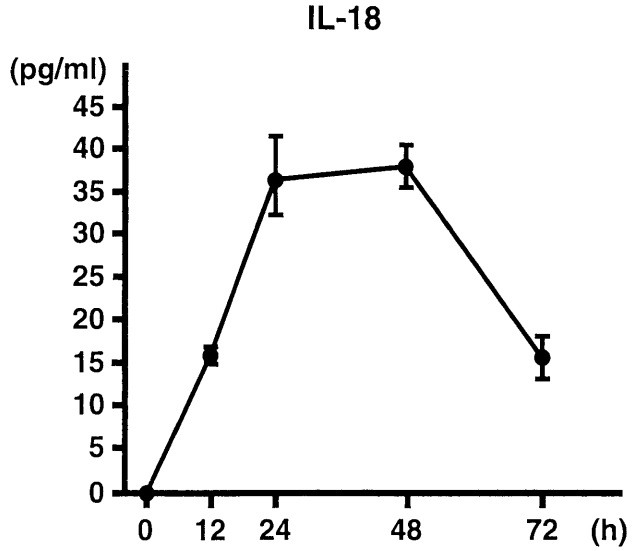

Fig. 1 Kinetics of IL-18 production in MLR.
Closed circles show the levels of IL-18 in pooled nupersatants from MLR. Values of IL-18 are expressed as pg/ml. Assays were performed in triplicate, and values are means ± SEM.

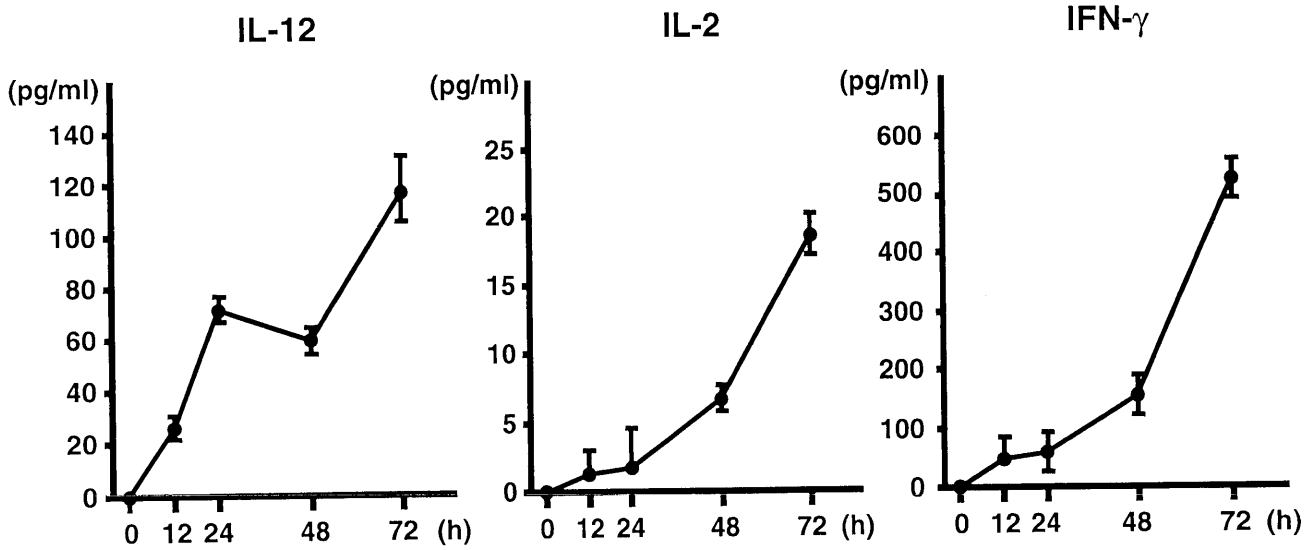

Fig. 2 Kinetics of Th1 cytokine productions in MLR.
Closed circles show the levels of cytokines in pooled supernatants from MLR. Values of IL-12, IL-2 and IFN-γ are expressed as pg/ml. Assays were performed in triplicate, and values are means ± SEM.

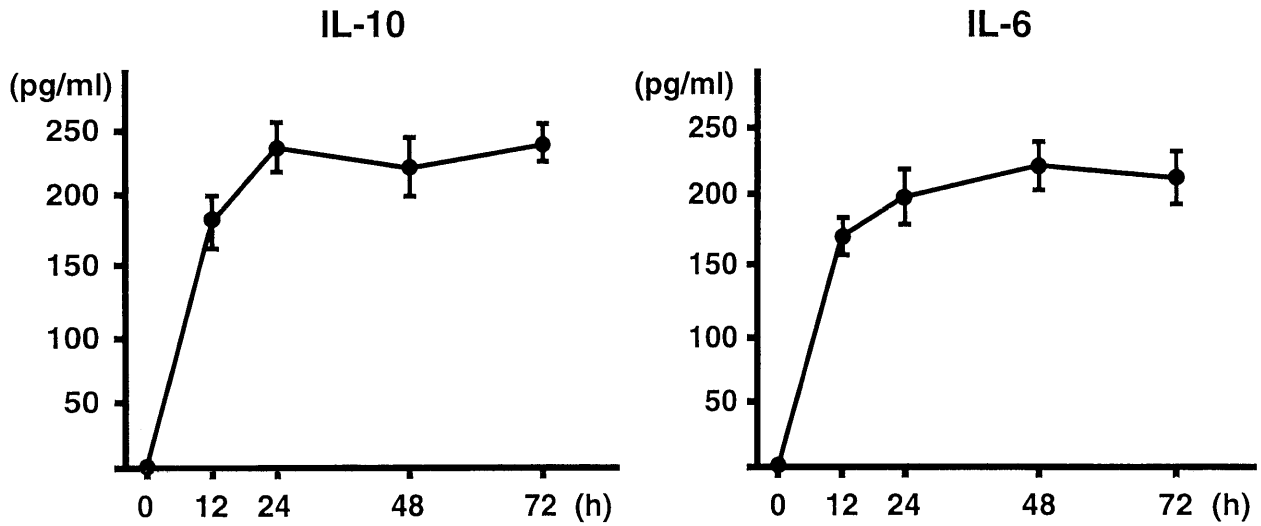

Fig. 3 Kinetics of Th2 cytokine productions in MLR.
Closed circles show the levels of cytokines in pooled supernatants from MLR. Values of IL-10 and IL-6 are expressed as pg/ml. Assays were performed in triplicate, and values are means ± SEM.

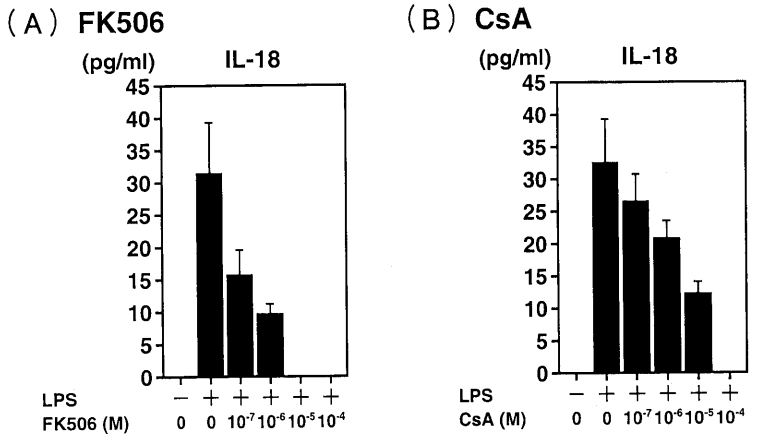

Fig. 4 Effect of FK506/CsA on IL-18 production in MLR.
FK506 and CsA were added to the media at the start of incubation and cultured for 48 h. Assays were performed in triplicate, and values are mean ± SEM.

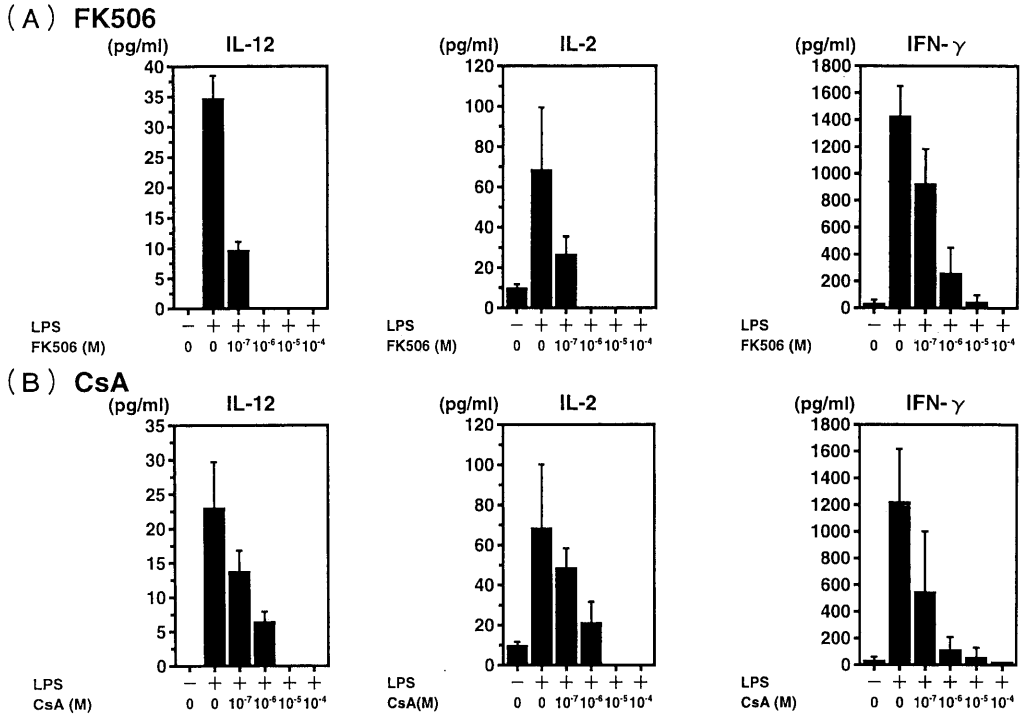

Fig. 5 Effect of FK506/CsA on Th1 cytokine productions in MLR.
FK506 and CsA were added to the media at the start of incubation and cultured for 48 h. Assays were performed in triplicate, and values are mean ± SEM.

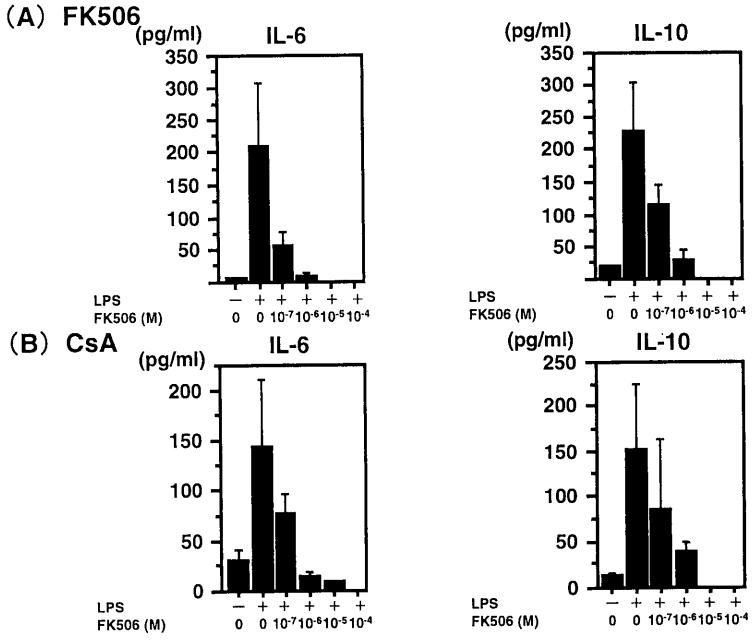

Fig. 6 Effect of FK506/CsA on Th2 cytokine productions in MLR.
FK506 and CsA were added to the media at the start of incubation and cultured for 48 h. Assays were performed in triplicate, and values are mean ± SEM.

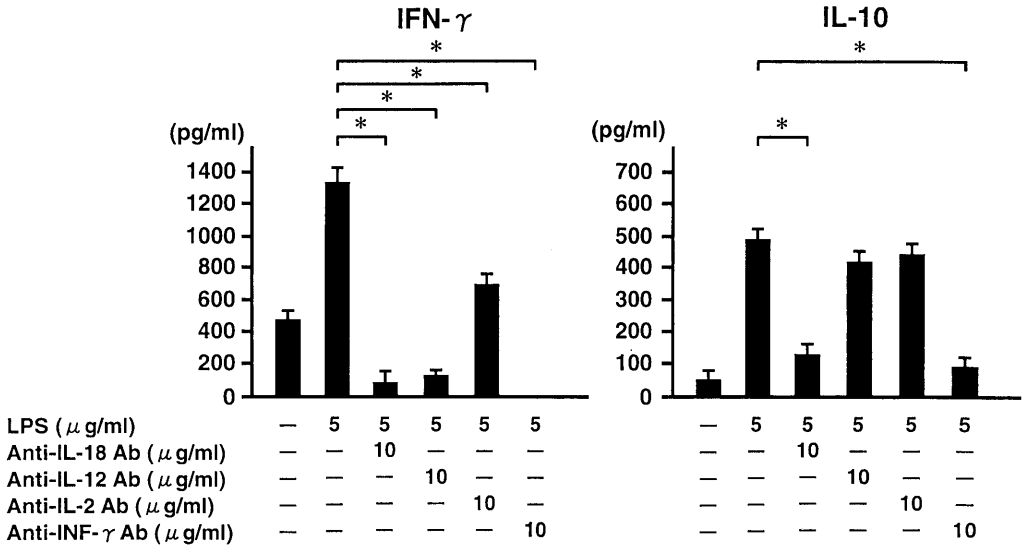

Fig. 7 Blocking test in MLR.
MLR was treated with anti-IL-18 Ab, anti-IL-12 Ab, anti-IL-2 Ab and anti-IFN-γ Ab for 48 h. The assay was performed in triplicate, and values represent means ± SEM. *$P < 0.01$ versus Ab untreated.

Discussion

FK-506 and CsA are calcineurin antagonists. These agents act, in part, through the inhibition of nuclear transcription factors such as NFAT and NF-κB. Functionally, FK-506 and CsA inhibit the transcription of numerous cytokines and immunomodulators involved in T-cell activation and proliferation (8, 9). Previous studies have demonstrated the ability of calcineurin antagonists to downregulate IFN-γ (12–15). However, cellular activation in these studies was achieved with mitogens; therefore, the relevance of these systems to antigen-presenting cell (APC) and antigen-driven Th1 and Th2 phenotypic responses is unclear.

The MLR is an important *in vitro* model for studying alloresponsiveness. Using the MLR, we can measure the disparity in major histocompatibility complex (MHC) antigens between individuals (16, 17). Because cytokines play a crucial role in the posttransplantation response, cytokine release and interactions in MLR are important in the field of transplantation medicine. In human MLR, responder cells recognize alloantigens on the surface of stimulator cells and undergo proliferation. It has been shown that IFN-γ is secreted predominantly into the supernatant and that the IFN-γ response depends on differences in HLA-DR between the 2 individuals (18, 19).

The current study showed that production of IL-18 was upregulated at 12 h, reached a near maximum level at 24 h, and decreased at 72 h. The decrease of IL-18 at 72 h might be attributable to its consumption and degradation or to the negative effect on the IL-18-initiating cytokine cascade caused by the accumulation of Th2 cytokines. Production of both IL-12 and IL-2 was observed at 12 h and then increased markedly. Production of IFN-γ was detected at 12 h and increased there after (Fig. 1, Fig. 2). These results suggested that production of IFN-γ depends on IL-18, IL-12 and IL-2 in the early phase (12–48 h) and predominantly on both IL-12 and IL-2 in the late phase (48–72 h) of the human MLR system.

The synergistic actions of IL-18 and IL-12 have been observed in the production of IFN-γ (20–23). Yoshimoto *et al.* demonstrated that IL-12 upregulated the expression of IL-18 receptors in T cells, Th1 cells and B cells, which enabled the synergistic production of IFN-γ (21). In contrast, IL-18 can stimulate IFN-γ production in an IL-12-independent manner in KG-1, a monocytic cell line (8). It is quite likely that the cooperative coexistence of IL-18 and IL-12 can induce a strong Th1 response in the human MLR system.

The potential differential regulation of human antigen-stimulated T cell subsets by calcineurin antagonists has not been fully described. The present research has demonstrated concentration-dependent inhibition by calcineurin antagonists of IL-18, Th1 and Th2 cytokine productions in the MLR system (Fig. 4, Fig. 5, Fig. 6). These results suggested that calcineurin antagonists

promoted an equivalent down-regulation of Th1 and Th2 responses in the human MLR system. However, a number of reports have suggested a relative resistance of Th2 cytokines to the effects of calcineurin antagonists (24–35). Differences in cell populations and methods of cellular activation used in these experiments complicate their interpretation and are likely to account for the contradictory results.

It has been reported that mitogenic stimulation of phenotypically specific $CD4^+$ $CD4^+$ T cell clones results in nonphenotypically selective cytokine generation; however, subsequent restimulation with antigen and antigen-presenting cells (APCs) will restore phenotypic specificity (36, 37). Thus, mitogen stimulation appears to obviate phenotypic specificity. Moreover, mitogen stimulation is clearly less physiologic than antigen stimulation. Finally, it has been reported that sensitivity to calcineurin antagonists may vary with the quantity of the activation signal applied to the responder cells (38).

We also found differences in cytokine production profiles of IFN-γ and IL-10 between IL-18 and other cytokines in the MLR system. In the blocking test, the addition of anti-IL-18 Ab and anti-IFN-γ Ab significantly ($P < 0.01$) reduced the production of IFN-γ and IL-10. In contrast, treatment with anti-IL-12 Ab and anti-IL-2 Ab significantly ($P < 0.01$) inhibited IFN-γ production but not IL-10 production, as shown in Fig. 5. These results suggested that IL-18 and IFN-γ play a role in the induction of IL-10, which may function as a negative feedback for excessive Th1 response in the MLR system.

In contrast, IL-12 and IL-2 do not induce IL-10 directly, at least in the MLR system. It has also been reported that treatment with IL-18 and anti-CD3 Ab produced IL-2 and IFN-γ in Th1 cells (39, 40). IL-18 strongly induced NF-κB activation, which was detected by electrophoretic mobility shift assay using an NF-κB binding site of the IL-2 promoter and reporter gene analysis in Th1 cells (41). Taking these results into consideration, it is likely that IL-18 is an important triggering cytokine in allogeneic cell interactions and is present in the most upstream of cytokine cascades in immune responses, at least in the MLR system.

IL-12 has also reportedly induced IFN-γ production by Th1 cells, and the immune responses induced by IL-12 are similar to those induced by IL-18 *in vivo* (41). However, the signals for IFN-γ production by IL-18 have been revealed to be different from those induced by IL-12, since IL-18 with IL-12 synergistically induced IFN-γ production (42), which was consistent with the results of the present study. As a unique signal activated by IL-12, it was reported that STAT4 was essential for IFN-γ production. Furthermore, it has been reported that IL-18 could not induce STAT4 activation in Th1 cells (7). A recent report suggested that dual signaling consisting of IL-18-induced NF-κB activation and TCR/CD3-mediated NFAT activation is crucial for IFN-γ production in Th1 cells (43).

As described above, IL-18 is secreted from LPS-activated macrophages. Although LPS alone did not induce IL-18 production in PBMCs (9), the combination of the LPS/MLR system dramatically induced IL-18, as shown in the present study. Furthermore, calcineurin antagonists inhibited IL-18 production dose-dependently in human antigen-stimulated PBMCs. These results suggested that dual signaling consisting of LPS-induced NF-kB activation and antigen-driven NFAT activation is crucial for IL-18 production in macrophages.

In conclusion, the present study clearly demonstrated the importance of IL-18 in allogeneic cell interactions and that its generation in the human MLR system was strongly inhibited by either FK-506 or CsA. Although there is evidence that calcineurin antagonists can effect T cell responses through interference with antigen presentation from APCs (44), the similarity of PBMCs in the MLR system of this study militate against significant confounding of these data. The current study also suggested that dual signaling consisting of antigen-driven NFAT activation and LPS-mediated NF-kB activation is crucial for IL-18 production in monocytes/macrophages.

Recent research has clarified the mechanism of Caspase-1-independent, Fas/Fas ligand-mediated IL-18 secretion from macrophages. Administration of Propionibacterium acnes upregulated functional Fas expression of macrophages in an IFN-γ-dependent manner, and these macrophages became able to secrete mature IL-18 upon stimulation with FasL (45). However, which molecules induce IL-18 has not yet been fully elucidated. Further research is warranted for elucidating the production of IL-18.

References

1. Okamura H, Tsutsui H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, Torigoe K, Okura T, Nukada Y, Hattori K, Akita K, Namba M, Tanabe F, Konishi K, Fukuda S and Kurimoto M: Cloning of a new cytokine that induces IFN-γ production by T cells. Nature (1995) **378**, 88–91.

2. Kohno K and Kurimoto M: .Interleukin 18, a cytokine which resembles IL-1 structurally and IL-12 functionally but exerts its effect independently of both. Clin Immunol Immunopathol (1998) **86**, 11-15.
3. Dinarello CA: IL-18: A TH1-inducing, proinflammatory cytokine and new member of the IL-1 family. J Allergy Clin Immunol (1999) **103**, 11-24.
4. Micallef MJ, Ohtsuki T, Kohno K, Tanabe F, Ushio S, Namba M, Tanimoto T, Torigoe K, Fujii M, Ikeda M, Fukuda S and Kurimoto M: Interferon-gamma-inducing factor enhances T helper I cytokine production by stimulated human T cells: Synergism with interleukin-12 for interferon-gamma production. Eur J Immunol (1996) **16**, 1647-1651.
5. Ushio S, Namba M, Okura T, Hattori K, Nukada Y, Akita K, Tanabe F, Konishi K, Micallef M, Fujii M, Torigoe K, Tanimoto T, Fukuda S, Ikeda M, Okamura H and Kurimoto M: Cloning of the cDNA for human IFN-gamma-inducing factor, expression in Escherichia coli, and studies on the biologic activities of the protein. J Immunol (1996) **156**, 4274-4279.
6. Kohno K, Kataoka J, Ohtsuki T, Suemoto Y, Okamoto I, Usui M, Ikeda M and Kurimoto M: IFN-gamma-inducing factor (IGIF) is a costimulatory factor on the activation of Th1 but not Th2 cells and exerts its effect independently of IL-12. J Immunol (1997) **158**, 1541-1550.
7. Robinson D, Shibuya K, Mui A, Zonin F, Murphy E, Sana T, Hartley SB, Menon S, Kastelein R, Bazan F and O'Garra A: IGIF does not drive TH1 development but synergizes with IL-12 for interferon-gamma production and activates IRAK and NFkappaB. Immunity (1997) **7**, 571-581.
8. Kohka H, Yoshino T, Iwagaki H, Sakuma I, Tanimoto T, Matsuo Y, Kurimoto M, Orita K, Akagi T and Tanaka N: Interleukin-18/interferon-γ-inducing factor, a novel cytokine, up-regulates ICAM-1 (CD54) expression in KG-1 cells. J Leuk Biol (1998) **64**, 519-527.
9. Kohka H, Iwagaki H, Yoshino T, Kobashi K, Urushihara N, Yagi T, Tanimoto T, Kurimoto M, Akagi T and Tanaka N: Involvement of Interleukin-18 (IL-18) in Mixed Lymphocyte Reactions (MLR). J Interferon Cytokine Res (1999) **19**, 1053-1057.
10. Gardenas ME, Zhu D and Heitman J: Molecular mechanisms of immunosuppression by cyclosporin, FK506 and rapamycin. Curr Opin Nephrol Hypertens (1995) **4**, 472-477.
11. Mattila PS: The actions of cyclosporin A and FK506 on T-lymphocyte activation. Biochem Soc Trans (1996) **24**, 45-49.
12. Batiuk TD, Pazderka F, Enns J, DeCastro L and Halloran PF: Cyclosporine inhibition of calcineurin activity in human leukocytes *in vivo* is rapidly reversible. J Clin Invest (1995) **96**, 1254-1260.
13. Batiuk TD, Pazderka F, Enns J, De Castro L and Halloran PF: Cyclosporine inhibition of leukocyte calcineurin is much less in whole blood than in culture medium. Transplantation (1996) **61**, 158-161.
14. Briscoe DM, Henault LE, Geehan C, Alexander SI and Lichtman AH: Human endothelial cell costimulation of T cell IFN-gamma production. J Immunol (1997) **159**, 3247-3256.
15. Iacono A, Dauber J, Keenan R, Spichty K, Cai J, Grgurich W, Burckart G, Smaldone G, Pham S, Ohori NP, Yousem S, Williams P, Griffith B and Zeevi A: Interleukin-6 and interferon-gamma gene expression in lung transplant recipients with refractory acute cellular rejection: Implications for monitoring and inhibition by treatment with aerosolized cyclosporine. Transplantation (1997) **64**, 263-269.
16. Baib B, Vas MR and Lowenstein L: The development of large immature mononuclear cells in mixed leukocyte cultures. Blood (1964) **23**, 271-275.
17. Bach FH and Voynow NK: One-way stimulation in mixed leukocyte cultures. Science (1966) **153**, 545-547.
18. Perussia B, Mangoni L, Engers HD and Trinchieri G: Interferon production by human and murine lymphocytes in response to alloantigens. J Immunol (1980) **125**, 1589-1595.
19. Cantell K, Koskimies S and Hirvonen S: Production of interferon in mixed leukocyte cultures from two individuals. J Interferon Res (1990) **10**, 331-335.
20. Okamura H, Kashiwamura S, Tsutsui H, Yoshimoto T and Nakanishi K: Regulation of interferon-gamma production by IL-12 and IL-18. Curr Opin Immunol (1998) **10**, 259-264.
21. Yoshimoto T, Takeda K, Tanaka T, Ohkusu K, Kashiwamura S, Okamura H, Akira S and Nakanishi K: IL-12 up-regulates IL-18 receptor expression on T cells, Th1 cells, and B cells: Synergism with IL-18 for IFN-gamma production. J Immunol (1998) **161**, 3400-3407.
22. Munder M, Mallo M, Eichmann K and Modolell M: Murine macrophages secrete interferon gamma upon combined stimulation with interleukin (IL)-12 and IL-18: A novel pathway of autocrine macrophage activation. J Exp Med (1998) **187**, 2103-2108.
23. Rothe H, Jenkins NA, Copeland NG and Kolb H: Active stage of autoimmune diabetes is associated with the expression of a novel cytokine, IGIF, which is located near Idd2. J Clin Invest (1997) **99**, 469-474.
24. Abbas AK, Murphy KM and Sher A: Functional diversity of helper T lymphocytes. Nature (1996) **383**, 787-793.
25. Tian L, Stepkowski SM, Qu X, Wang ME, Wang M, Yu J and Kahan BD: Cytokine mRNA expression in tolerant heart allografts after immunosuppression with cyclosporine, sirolimus or brequinar. Transpl Immunol (1997) **5**, 189-198.
26. Lang T, Krams SM, Villanueva JC, Cox K, So S, Esquivel C and Martinez OM: Distinct patterns of Th2 cytokine production during immune activation in pediatric liver allograft recipients. Transplant Proc (1995) **27**, 1146-1147.
27. Takamatsu Y, Hasegawa M, Sato S and Takehara K: IL-13 production by peripheral blood mononuclear cells from patients with atopic dermatitis. Dermatology (1998) **196**, 377-381.
28. Homey B, Assmann T, Vohr HW, Ulrich P, Lauerma AI, Ruzicka T, Lehmann P and Schupper HC: Topical FK506 suppresses cytokine and costimulatory molecule expression in epidermal and local draining lymph node cells during primary skin immune responses. J Immunol (1998) **160**, 5331-5340.
29. Dolganov G, Bort S, Lovett M, Burr J, Schubert L, Short D, Mogurn M, Gibson C and Lewis DB: Coexpression of the interleukin-13 and interleukin-4 genes correlates with their physical linkage in the cytokine gene cluster on human chromosome 5q23-31. Blood (1996) **87**, 3316-3326.
30. Rafiq K, Kasran A, Peng X, Warmerdam PA, Coorevits L, Ceuppens JL and Van Gool SW: Cyclosporin A increases IFN-gamma production by T cells when co-stimulated through CD28. Eur J Immunol (1998) **28**, 1481-1491.
31. Redrup AC, Howard BP, MacGlashan DW Jr, Kagey-Sobotka A, Lichtenstein LM and Schroeder JT: Differential regulation of IL-4 and IL-13 secretion by human basophils: Their relationship to histamine release in mixed leukocyte cultures. J Immunol (1998) **160**, 1957-1964.
32. Dumont FJ: FK506 enhances IL-13 production by T cells activated through CD3/CD28. Int Arch Allergy Immunol (1997) **114**, 300-301.
33. van der Pouw Kraan TC, Boeije LC, Troon JT, Rutschmann SK, Wijdenes J and Aarden LA: Human IL-13 production is negatively influenced by CD3 engagement, enhancement of IL-13 production by cyclosporin A. J Immunol (1996) **156**, 1818-1823.
34. Mori A, Suko M, Nishizaki Y, Kaminuma O, Kobayashi S, Matsuzaki G, Yamamoto K, Ito K, Tsuruoka N and Okudaira H: IL-5 production by CD4+ T cells of asthmatic patients is suppressed by glucocorticoids and the immunosuppressants FK506 and cyclosporin A. Int

Immunol (1995) **7**, 449–457.
35. Valentine JE and Sewell WA: Induction of IL-5 expression by IL-2 is resistant to the immunosuppressive agents cyclosporine A and rapamycin. Int Immunol (1997) **9**, 975–982.
36. Essayan DM, Han WF, Li XM, Xiao HQ, Kleine-Tebbe J and Huang SK: Clonal diversity of IL-4 and IL-13 expression in human allergen-specific T lymphocytes. J Allergy Clin Immunol (1996) **98**, 1035–1044.
37. Gajewski TF, Schell SR and Fitch FW: Evidence implicating utilization of different T cell receptor-associated signaling pathways by TH1 and TH2 clones. J Immunol (1990) **144**, 4110–4120.
38. Pazderka F, Enns J, Batiuk TD and Halloran PF: The functional consequences of partial calcineurin inhibition in human peripheral blood mononuclear leucocytes. Transpl Immunol (1996) **4**, 23–31.
39. Matsumoto S, Tsuji-Takayama K, Aizawa Y, Koide K, Takeuchi M, Ohta T and Kurimoto M: Interleukin-18 activates NF-kappaB in murine T helper type 1 cells. Biochem Biophys Res Commun (1997) **234**, 454–457.
40. Tsuji-Takayama K, Matsumoto S, Koide K, Takeuchi M, Ikeda M, Ohta T and Kurimoto M: Interleukin-18 induces activation and association of p56 (lck) and MAPK in a murine TH1 clone. Biochem Biophys Res Commun (1997) **237**, 126–130.
41. Ahn HJ, Maruo S, Tomura M, Mu J, Hamaoka T, Nakanishi K, Clark S, Kurimoto M, Okamura H and Fujikawa H: A mechanism underlying synergy between IL-12 and IFN-gamma-inducing factor in enhanced production of IFN-gamma. J Immunol (1997) **159**, 2125–2131.
42. Kohno K, Kataoka J, Ohtsuki T, Suemoto Y, Okamoto I, Usui M, Ikeda M and Kurimoto M: IFN-gamma-inducing factor (IGIF) is a costimulatory factor on the activation of Th1 but not Th2 cells and exerts its effect independently of IL-12. J Immunol (1997) **158**, 1541–1550.
43. Tsuji-Takayama K, Aizawa Y, Okamoto I, Kojima H, Koide K, Takeuchi M, Ikegami H, Ohta T and Kurimoto M: Interleukin-18 induces interferon-gamma production through NF-kappaB and NFAT activation in murine T helper type 1 cells. Cell Immunol (1999) **196**, 41–50.
44. Little RG IInd, Ebertowski LA and David CS: Inhibition of alloantigen presentation by cyclosporine. Transplantation (1990) **49**, 937–944.
45. Tsutsui H, Kayagaki N, Kuida K, Nakano H, Hayashi N, Takeda K, Matsui K, Kashiwamura S, Hada T, Akira S, Yagita H, Okamura H and Nakanishi K: Caspase-1-independent, Fas/Fas ligand-mediated IL-18 secretion from macrophages causes acute liver injury in mice. Immunity (1999) **11**, 359–367.

Received April 27, 2000; accepted June 9, 2000.