Inducible Nitric Oxide Synthase Inhibition by Mycophenolic Acid

Dj. Miljkovic*[1], M. Markovic[2] and V. Trajkovic[2]

[1]*Institute for Biological Research, Belgrade, Serbia and Montenegro*

[2]*Institute of Microbiology and Immunology, School of Medicine, Belgrade, Serbia and Montenegro*

Abstract: The focus of this review is the influence of an immunosuppressive xenobiotic drug mycophenolic acid on the induction of nitric oxide production in various cell types. The potential therapeutic significance of the cell-specific fine-tuning of nitric oxide release by mycophenolic acid, as well as the mechanisms behind the drug action are discussed.

Keywords: Mycophenolic acid, mycophenolate mofetil, nitric oxide, iNOS.

INTRODUCTION

Immunosuppressive Action of Mycophenolic Acid

Mycophenolic acid (MPA) is the principal bioactive compound of mycophenolate mofetil (MMF), an immunosuppressive xenobiotic drug, which has been used in preventing transplant rejection [43]. MMF was also shown efficient in the treatment of various immune-mediated diseases in animal models [3], as well as of rheumatoid arthritis [3, 52] and autoimmune skin disorders in humans [13, 21, 30, 31].

MPA inhibits the activity of inosine monophosphate dehydrogenase (IMPDH), a rate-limiting enzyme for *de novo* synthesis of guanosine nucleotides (Fig. (**1**)). By depleting the intracellular concentration of guanosine nucleotides, MPA acts as a powerful proliferation inhibitor in various cell types, especially in activated lymphocytes [3]. This selectivity is a consequence of its preference for IMPDH isoform II, which is expressed in lymphocytes upon their activation [36, 48]. Apart from proliferation, MPA inhibits cytokine production, glycosylation and expression of adhesion molecules in cultured human T cells, as well as antibody formation of *in vitro* activated human B lymphocytes [1, 2, 16, 18]. MPA was also shown effective i*n vivo*, since it inhibited both primary and secondary humoral response in rodents [1, 22, 23] and humans [3].

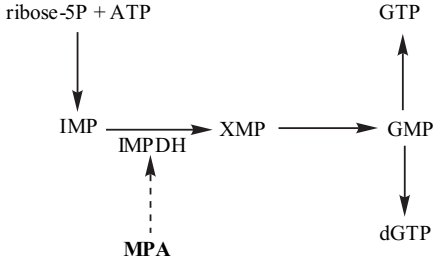

Fig. (1). Interference of MPA with *de novo* guanosine nucleotide synthesis. ATP - adenosine monophosphate; IMP - inosine monophosphate; XMP - xanthosine monophosphate; GMP - guanosine monophosphate; GTP - guanosine triphosphate; dGTP - deoxyguanosine triphosphate; IMPDH - inosine monophosphate dehydrogenase.

*Address correspondence to this author at the Institute for Biological Research, (Department for Neurobiology and Immunology) 29. Novembra 142, Belgrade, Serbia and Montenegro; Tel/Fax: ++381 11 2657-258; E-mail: georgije_zw@yahoo.com

There is a growing body of evidence suggesting a potent influence of MPA on cell types other than lymphocytes. MPA significantly reduced cytokine-induced expression of selectins and VCAM-1, together with prostaglandin E2 synthesis in endothelial cells [5, 8, 9, 10, 50]. On the other hand, the ICAM-1 expression on human umbilical vein endothelial cells (HUVEC) was augmented by its action [59]. Importantly, MPA was shown capable of impairing maturation and differentiation of murine dendritic cells, as well as their ability to stimulate allogeneic T cells [42]. Therefore, besides directly acting on lymphocytes, MPA might exert immunoregulatory properties through influencing the function of other cells involved in the immune response.

The Role of Nitric Oxide in Immunity

Nitric oxide (NO) is synthesized from L-arginine by intracellular enzyme NO synthase (NOS). Neuronal (nNOS, NOS1) and endothelial (eNOS, NOS3) NOS isoforms are constitutively expressed and generate low amount of NO involved in regulation of neurotransmission and vascular homeostasis, respectively [11, 39]. In contrast, the expression of inducible isoform (iNOS, NOS2) is induced during immune response in macrophages, endothelial cells, fibroblasts, astrocytes, vascular smooth muscle cells and other cell types in reaction to the microbial products and/or cytokines [12]. High-output NO production by iNOS is one of the major microbicidal and tumoricidal mechanisms of the immune system [12, 39]. However, NO could be involved as an effector molecule in excessive tissue destruction during inappropriate immune response occurring in prolonged infections, in diseases with autoimmune etiology or in hypersensitivity reactions [11]. Alternatively, NO also has a profound immunomodulatory property, including suppression of T lymphocyte and antigen-presenting cell function [12, 37].

THE INFLUENCE OF MPA ON INOS-MEDIATED NO PRODUCTION

There are several reports concerning MPA's effect on NO production in various cell types, both *in vivo* and *in vitro*. Although all i*n vivo* experiments on animals were conducted with MMF, the observed effects of MMF in these experiments could be fairly attributed to MPA, since MPA is its only known active metabolite [3].

1389-5575/04 $45.00+.00

MPA Modulation of NO Production *in vivo*

It is well appreciated that excessive production of TNF-α and NO contributes to inflammation and cardiovascular collapse seen in endotoxic shock [6, 27]. Pretreatment of LPS-injected animals with MMF inhibited the release of TNF-α and NO, promoted production of IL-10, and protected mice from LPS-induced lethality [20]. Interestingly, MMF pretreatment did not alter NO production in IL-10 deficient mice injected with LPS, thus suggesting that the upregulation of IL-10 synthesis participates in the drug-mediated suppression of NO release [20]. This is consistent with the proposed inhibitory effect of this anti-inflammatory cytokine on NO synthesis in sepsis [17, 63]. Similar results were obtained in streptozotocin-induced diabetes in mice, an autoimmune disease characterized by NO-mediated killing of pancreatic β-cells [40]. MMF treatment of diabetic animals augmented IL-10 production and down-regulated NO release from peritoneal macrophages, which might partly account for the protective effect of the drug in this disease [40].

The influence of MMF on iNOS-mediated NO production was also investigated in the mouse model of renal ischemia-reperfusion injury (IRI) [38]. While only low levels of NO and iNOS mRNA were detected in the sham-operated kidneys, they were significantly increased in mice with renal IRI. However, both the expression of iNOS gene and subsequent NO release were reduced in a dose-dependent fashion by pre-treatment with MMF. Overexpression of iNOS with high NO production in kidneys is also a hallmark of lupus glomerulonephritis in systemic lupus erithemathosus (SLE) in humans [60]. Mice of MRL strain that are homozygous for the defective Fas gene, called lpr (MLR/lpr mice), spontaneously develop a lupus-like syndrome, and are frequently used as a model for human SLE. It was recently reported that renal cortical iNOS mRNA level and urinary nitrite production were markedly reduced in MLR/lpr mice treated with MMF. Furthermore, this effect was accompanied by significant reduction of glomerulonephritis signs, such as glomerulosclerosis, glomerular volume and proteinuria.

MPA Modulation of NO Production *in vitro*

So far, MPA influence on iNOS-mediated NO production *in vitro* was investigated in rat or mouse endothelial cells, astrocytes, fibroblasts and macrophages, and the results of these studies are summarized in Table 1.

Endothelial Cells

In rat brain vascular endothelial cells, MPA inhibited NO production induced by combination of IFN-γ and TNF-α [53]. However, MPA did not affect low basal NO synthesis in unstimulated cells, suggesting that iNOS, rather than constitutive NOS (cNOS or eNOS), was the target for the drug action [53]. Interestingly, although the regulation of iNOS is mainly transcriptional [12, 39], MPA effect in endothelial cells was apparently exerted through suppression of iNOS co-factor tetrahydrobiopterin (BH_4). BH_4 is essential for optimal functioning of iNOS enzyme [12, 39] and its intracellular concentration is controlled by *de novo* synthesis, mainly by the feedback regulation of GTP hydrolase I [26, 28, 29]. As the substrate for GTP hydrolase I is GTP, the synthesis of BH_4 is dependent on IMPDH activity. Therefore, MPA-imposed inhibition of IMPDH activity could lead to restricted levels of BH_4 in cells and subsequent impairment of iNOS catalytic activity. The addition of the direct BH_4 precursor sepiapterin to MPA-treated endothelial cells completely restored their NO release, thus arguing in favor of such an assumption. Exogenous guanosine, which overcomes IMPDH block by acting as a salvage pathway precursor for GTP synthesis, completely

Table 1. The Influence of MPA on iNOS-Mediated NO Release *In vitro*. All Cells Described in the Table are of Rat Origin, Except Mouse Cell Lines L929 and IC-21

Cell type	iNOS stimulus	NO production	iNOS expression	iNOS catalytic activity	Ref.
Endothelium					
Brain vascular endothelial cells	IFN-γ/TNF-α	↓	na	↓	[53]
Astrocytes					
Primary astrocytes	IFN-γ/LPS	↓	↓	Ø	[45]
C6 astrocytoma	IFN-γ/LPS	↓	na	na	[45]
Fibroblasts					
Primary fibroblasts	IFN-γ/LPS	↓	↓	Ø	[47]
L929 fibrosarcoma	IFN-γ/LPS	↓	na	na	[46]
Macrophages					
Peritoneal cells	IFN-γ/LPS	Ø	Ø	na	[45, 47]
IC-21 cell line	IFN-γ/LPS	↓	↓	na	[34]

abolished the effect of MPA on endothelial NO release [45, 53], thus confirming that the drug acted through IMPDH inhibition.

Astrocytes and Fibroblasts

In contrast to the results obtained with rat endothelial cells, sepiapterin failed to prevent drug inhibitory action on IFN-γ + LPS-induced NO synthesis in rat primary astrocytes or fibroblasts [45, 47]. Hence, MPA effect in these cells did not stem from the limitation of BH_4 availability and resulting suppression of iNOS enzymatic activity. This discrepancy was not a consequence of different iNOS stimuli applied in the two studies, as sepiapterin was also unable to revert the MPA inhibitory action in astrocytes and fibroblasts treated with IFN-γ + TNF-α. The observed block of NO production in astrocytes and fibroblasts rather involved the interference of MPA with IFN-γ + LPS-triggered expression of iNOS gene, as supported by MPA's inability to affect NO production if transcription was blocked, as well as by the reduced expression of iNOS mRNA in MPA-treated cells. However, MPA obstruction of iNOS catalytic activity in astrocytes or fibroblasts could not be completely ruled out, since such an ability of the drug would be presumably preceded and, therefore, masked by its interference with iNOS gene expression. In fact, this notion is supported by the finding that MPA-mediated suppression of NO production was more prominent than that of iNOS mRNA expression. Interestingly, MPA failed to inhibit astrocyte or fibroblast iNOS activation in the presence of guanosine, thus strongly indicating that the drug effect was completely dependent on the down-regulation of IMPDH activity. The inhibitory action of MPA on iNOS-mediated NO release was not restricted to primary cells, as it was also observed in rat astrocytoma line C6 and mouse fibrosarcoma cell line L929 [45, 47].

Macrophages

MPA significantly down-regulated both TNF-α and NO release in IFN-γ + LPS-stimulated cultures of murine macrophage cell line IC-21 [34]. However, these effects were apparently due to IMPDH inhibition-dependent cytotoxic action of the drug. On the other hand, both IFN-γ and/or LPS-activated rat and mouse primary macrophages were absolutely resistant to MPA toxic action observed in their transformed counterparts, as well as to the drug interference with iNOS expression or catalytic activity that was operative

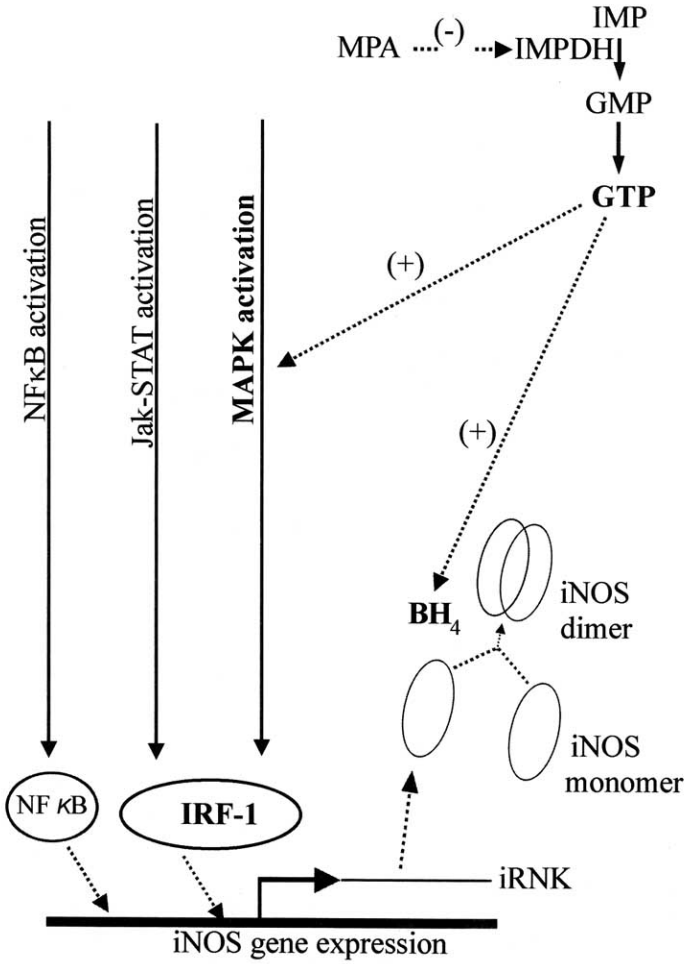

Fig. (2). The influence of MPA on iNOS mediated NO production. Several signaling pathways, including Jak-STAT and MAPK, are involved in the activation of the essential iNOS transcription factor IRF-1 and subsequent transcription of the iNOS gene. Decrease of GTP intracellular concentration by MPA could interfere with MAPK-induced IRF-1 activation, as well as with the synthesis of BH_4, an indispensable iNOS co-factor.

in fibroblasts/astrocytes, or endothelial cells, respectively. This is unlikely to be due to macrophage insensitivity to MPA-mediated IMPDH inhibition, since MPA caused a rapid down-regulation of guanosine nucleotide content in monocytes [3]. While possible difference in BH_4 availability might account for distinct MPA effect on the enzymatic activity of macrophage and endothelial iNOS, the absence of MPA influence on the expression of macrophage iNOS indicates that intracellular pathways controlling iNOS activation in fibroblast or astrocytes and macrophages might differ. This finding also implies that down-regulation of NO release by MPA observed in sepsis and diabetes [20, 40] could not result from the direct inhibition of macrophage iNOS, but rather from alterations in cytokine network that controls iNOS expression. Indeed, in both studies the suppression of NO release correlated with reduced production of iNOS-activating proinflammatory cytokines TNF-α and/or IFN-γ, as well as with augmented synthesis of anti-inflammatory iNOS-deactivating cytokine IL-10 [20, 40].

Intracellular Mechanisms for MPA Inhibition of iNOS Induction

A coordinated binding of two transcription factors, NF-κB and IRF-1, to their consensus sequences in iNOS promoter, is necessary for optimal iNOS transcription [51] (Fig. (**2**)). Unlike NF-κB, which pre-exists in the cytoplasm in the inactive state, IRF-1 is activated mainly at the transcriptional level via Jak-triggered induction of transcriptional factor STAT1 [54]. Interestingly, a striking down-regulation of IRF-1 mRNA coincided with MPA suppression of iNOS gene expression in astrocytes and fibroblasts [45, 47], thus providing a plausible explanation for the drug effect. Moreover, this indicates that similar mechanism might be also responsible for the reduced renal iNOS mRNA expression in MPA-treated lupus mice, since the observed drug action was apparently independent of modulation of NF-κB pathway [64]. While LPS is a prototype NF-κB activator [25], IRF-1 is a principal mediator of IFN-γ intracellular actions [54]. It therefore, seems conceivable to assume that MPA might exert its inhibitory action on fibroblast and astrocyte iNOS expression mainly through interference with IFN-γ-derived signals. The finding that MPA-sensitive expression of iNOS in lupus mice [64] depends mostly on IL-12-induced IFN-γ [32] is also consistent with the putative interference of MPA with IFN-γ signal transduction and IRF-1 activation. The absence of MPA influence on IRF-1 expression in macrophages [47] further supports possible involvement of impaired IRF-1 induction in the drug-mediated block of iNOS transcription.

The observed inhibition of IRF-1 expression by MPA in both astrocytes and fibroblasts was completely prevented by exogenous guanosine [45, 47], indicating that the drug effect was mediated through suppression of IMPDH activity. While the assumption that IMPDH might be required for the optimal induction of IRF-1 and, subsequently, iNOS, is intriguing, there is a question of the intracellular mechanism behind this IMPDH involvement. It was recently reported that IMPDH inhibition was linked with reduced levels of GTP-associated G-protein p21ras and the subsequent impairment of G-protein-dependent activation of mitogen activated protein kinase (MAPK) signaling cascade [56]. The members of MAPK signaling pathways have recently been implicated in the induction of iNOS in fibroblasts and astrocytes [7, 19, 33, 44, 58], as well as IRF-1 in retinal epithelial cells or hepatocytes [24, 57]. Although the data on MAPK involvement in the iNOS induction in macrophages are somewhat conflicting, several studies suggested that induction of macrophage iNOS might be relatively insensitive to inhibition of MAPK, particularly to that of p44/42 MAPK [14, 15, 33, 44, 49]. Therefore, cell-selective down-regulation of iNOS gene expression by MPA might be achieved, at least in part, through IMPDH inactivation-dependent interference with MAPK signaling and subsequent block of IRF-1 induction (Fig. (**2**)).

CELL-SPECIFIC INHIBITION OF INOS BY MPA IN THE THERAPY OF AUTOIMMUNITY

Cytokine-activated iNOS expression and high NO production in both infiltrating macrophages and resident cells, may contribute to target tissue destruction in organ-specific autoimmune diseases. We will here try to postulate possible implications for the therapy of autoimmunity of the cell-specific MPA interference with the iNOS-mediated NO release *in vitro*.

It has become increasingly clear in recent years, that apart from its deleterious effect in the target tissue, NO might also limit the development of autoimmune T cells [11, 62]. This protective action in autoimmunity has been mainly ascribed to macrophages, which use NO to suppress the activation and clonal expansion of autoreactive T cells in lymphoid organs [37]. Indeed, peripheral macrophages were found to block the proliferation of encephalitogenic T cells in experimental allergic encephalomyelitis (EAE), an animal model of multiple sclerosis [61]. On the other hand, it has been shown that astrocytes, despite releasing high amounts of NO, use NO-independent mechanisms for the suppression of autoantigen-driven activation of T cells [45]. It is therefore, conceivable to assume that the interference of MPA with astrocyte, but not macrophage NO synthesis, might presumably preserve beneficial immunosuppressive effect of NO, while partly reducing its destructive action on the myelin sheath and neurons in the CNS. A similar line of reasoning can be employed for rheumatoid arthritis, where iNOS-mediated excessive release of NO by cytokine-activated synovial fibroblasts may contribute to joint cartilage destruction [4, 41]. Selective down-regulation of fibroblast iNOS by MPA might be sufficient to prevent excessive NO release and cell damage in the joint, while sparing presumably protective effect of macrophage NO at the periphery.

However, although *in vitro* results indicate that macrophages are fairly resistant to direct inhibition of NO release by MPA, it should be noted that macrophage NO production in autoimmune diabetes was significantly down-regulated by MPA treatment [40]. This might be a consequence of MPA interference with the production of T cell cytokines (IFN-γ, TNF-α) involved in activation of macrophage iNOS *in vivo* [40]. In the same study, MPA markedly reduced macrophage release of IL-12, which is a crucial cytokine involved in the development of Th1 response, while the production of anti-inflammatory Th2

cytokine IL-10 was increased [40]. Since Th1 cytokines (IL-12, IL-18, IL-2, IFN-γ) mainly promote, and Th2 cytokines (IL-4, IL-10, IL-13) repress iNOS induction [39], one could expect that shifting Th1/Th2 balance to the latter type of response might significantly contribute to MPA-mediated down-regulation of NO synthesis *in vivo*. However, such selective interference of MPA with Th1/Th2 cytokine production in autoimmunity was not universally observed, as the drug treatment suppressed both types of T cell response in experimental autoimmune encephalomyelitis and lupus-like disease in mice [35, 55].

CONCLUDING REMARKS

The studies presented here strongly indicate that novel immunosuppressant MPA has the ability to directly block iNOS-mediated NO release in different cell types, through mechanisms involving both interference with iNOS gene expression, as well as with iNOS catalytic activity. While iNOS induction or activity in macrophages was mainly refractory to MPA action *in vitro*, the drug might be able to suppress NO release *in vivo* by affecting the production of iNOS-inducing proinflammatory and/or Th1 cytokines. However, such indirect obstruction would presumably require some time to exert the effect, which at least during the initial period of the treatment, might leave intact NO-dependent immunosuppressive action of the macrophages. While the putative delay in inhibiting macrophage iNOS could be beneficial, immediate MPA-mediated direct blockade of iNOS in resident cells might contribute to the reduction of target tissue damage. It is on future studies to explore whether such time-dependent fine-tuning of NO production could be involved in the protective effects of MPA in autoimmunity and the treatment of transplant rejection.

REFERENCES

[1] Allison, A.C.; Almquist, S.J.; Muller, C.D.; Eugui, E.M. *Transplant. Proc.*, **1991,** *23*, 10.
[2] Allison, A.C.; Eugui, E.M. *Springer. Semin. Immunopathol.*, **1993,** *14*, 353.
[3] Allison, A.C.; Eugui, E.M. *Immunopharmacology,* **2000**, *47***,** 85.
[4] Amin, A.R.; Attur, M.; Abramson, S.B. *Curr. Opin. Rheumatol.*, **1999,** *11*, 202.
[5] Bertalanffy, P.; Dubsky, P.; Wolner, E.; Weigel, G. *Clin. Chem. Lab. Med.*, **1999,** *37*, 259.
[6] Beutler, B.; Milsark, I.W.; Cearmi, A.C. *Science*, **1985,** *229*, 869.
[7] Bhat, N.R.; Zhang, P.; Lee, J.C.; Hogan, E.L. *Neuroscience*, **1998,** *18*, 633.
[8] Blaheta, R.A.; Leckel, K.; Wittig, B.; Zenker, D.; Oppermann, E.; Harder, S.; Scholz, M.; Weber, S.; Schuldes, H.; Encke, A.; Markus, B.H. *Transpl. Immunol.*, **1998,** *6*, 251.
[9] Blaheta, R.A.; Leckel, K.; Wittig, B.; Zenker, D.; Oppermann, E.; Harder, S.; Scholz, M.; Weber, S.; Encke, A.; Markus, B.H. *Transplant. Proc.*, **1999,** *31*, 1250.
[10] Blaheta, R.A.; Nelson, K.; Oppermann, E.; Leckel, K.; Harder, S.; Cinatl, J.; Weber, S.; Shipkova, M.; Encke, A.; Markus, B.H. *Transplantation*, **2000,** *69*, 1977.
[11] Bogdan, C. *J. Exp. Med.*, **1998,** *187*,1361.
[12] Bogdan, C. *Nat. Immunol.*, **2001**, *2*, 907.
[13] Bohm, M.; Beissert, S.; Schwartz, T.; Metze, D.; Luger, T. *Lancet,* **1997,** *349*, 541.
[14] Caivano, M. *FEBS Lett.*, **1998,** *429*, 249.
[15] Chan, E.D.; Winston, B.W.; Uh, S.T.; Wynes, M.W.; Rose, D.M.; Riches, D.W. *J. Immunol.*, **1999,** *162*, 415.
[16] Chang, C-C.J.; Aversa, G.; Punnonen, J.; Yssel, H.; deVries, J. *Ann. N. Y. Acad. Sci.*, **1993,** *696*, 108.
[17] Chang, C.K.; Zdon, M.J. *Surg. Laparosc. Endosc. Percutan. Tech.,* **2002**, *12*, 247.
[18] Cohn, R.G.; Mirkovich, A.; Dunlap, B.; Burton, P.; Chiu, S.H.; Eugui, E.; Caulfield, J. P. *Transplantation*, **1999,** *68*, 411.
[19] Da Silva, J.; Pierrat, B.; Mary, J.L.; Lesslauer, W. *J. Biol. Chem.*, **1997,** *272*, 28373.
[20] Durez, P.; Appelboom, T.; Pira, C.; Stordeur, P.; Vray, B.; Goldman, M. *Int. J. Immunopharmacol.*, **1999,** *21*, 581.
[21] Enk, A.H.; Knop, J. *Arch. Dermatol.*, **1999,** *135*, 54.
[22] Eugui, E.M.; Almquist, S.; Muller; C.D.; Allison, A.C. *Scand. J. Immunol.*, **1991,** *33*, 161.
[23] Eugui, E.M.; Mircovich, A.; Allison, A.C. *Scand. J. Immunol.*, **1991,** *33*, 161.
[24] Faure, V.; Hecquet, C.; Courtois, Y.; Goureau, O. *J. Biol. Chem.*, **1999,** *274*, 4794.
[25] Ghosh, S.; May, M.J.; Kopp, E.B. *Annu. Rev. Immunol.*, **1998,** *16*, 225.
[26] Harada, T.; Kagamiyama, H.; Hatakeyama, K. *Science*, **1993,** *260*, 1507.
[27] Harbrecht, B.G.; Di Silvio, M.; Demetris, A.J.; Simmons, R.L.; Billiar, T.R. *Hepatology*, **1994,** *20*, 1055.
[28] Hatakeyama, K.; Harada, T.; Suzuki, S.; Watanabe, Y.; Kagamiyama, H. *J. Biol. Chem.*, **1989,** *264*, 21, 660.
[29] Hatakeyama, K.; Harada, T.; Kagamiyama, H. *J. Biol. Chem.*, **1992,** *267*, 20, 784.
[30] Haufs, M.G.; Beissert, S.; Grabbe, S.; Schutte, B.; Luger, T.A. *Br. J. Dermatol.*, **1998,** *138*, 179.
[31] Hohenleutner, U.; Mohr, V.D.; Michel, S.; Landthaler, M. *Lancet*, **1997,** *350*, 1748.
[32] Huang, F.P.; Feng, G.J.; Lindop, G.; Stott, D.I.; Liew, F.Y. *J. Exp. Med.*, **1996,** *83*, 1447.
[33] Jankovic, V.; Samardzic, T.; Stosic-Grujicic, S.; Popadic, D.; Trajkovic, V. *Cell. Immunol.*, **2000,** *199*, 73.
[34] Jonsson, C.A.; Carlsten, H. *Cell. Immunol.*, **2002**, *216*, 93.
[35] Jonsson, C.A.; Carlsten, H. *Clin. Exp. Immunol.*, **2001,** *124*, 486.
[36] Konno, Y.; Natsumeda, Y.; Nagai, M.; Yamaji, Y.; Ohno, S.; Suzuki, K.; Weber, G. *J. Biol. Chem.*, **1991**, *266*, 506.
[37] Liew, F.Y. *Curr. Opin. Immunol.*, **1995**, *7*, 396.
[38] Lui, S.L.; Chan, L.Y.; Zhang, X.H.; Zhu, W.; Chan, T.M.; Fung, P.C.; Lai, K.N. *Nephrol. Dial. Transplant.*, **2001**, *16*, 1577.
[39] MacMicking, J.; Xie, Q.; Nathan, C. *Ann. Rev. Immunol.*, **1997,** *10***,** 323.
[40] Maksimovic-Ivanic, D.; Trajkovic, V.; Miljkovic, D.J.; Stojkovic, M.M.; Stosic-Grujicic, S. *Clin. Exp. Immunol.*, **2002,** *129*, 214.
[41] McInnes, I.B.; Leung, B.P.; Field, M.; Wei, X.Q.; Huang, F.P.; Sturrock, R.D.; Kinninmonth, A.; Weidner, J.; Mumford, R.; Liew, F.Y. *J. Exp. Med.*, **1996,** *184*, 1519.
[42] Mehling, A.; Grabbe, S.; Voskort, M.; Schwarz, T.; Luger, T.A.; Beissert, S. *J. Immunol.*, **2000,** *165*, 2374.
[43] Mele, T.S.; Halloran, P.F. *Immunopharmacology*, **2000**, *47*, 215.
[44] Miljkovic, D.; Samardzic, T.; Mostarica Stojkovic, M.; Stosic-Grujicic, S.; Popadic, D.; Trajkovic, V. *Brain Res.*, **2001,** *889*, 331.
[45] Miljkovic, D.; Samardzic, T.; Cvetkovic, I.; Mostarica Stojkovic, M.; Trajkovic, V. *Glia*, **2002,** *39*, 247.
[46] Miljkovic, Dj.; Samardzic, T.; Drakulic, D.; Stosic-Grujicic, S.; Trajkovic, V. *Cytokine,* **2002**, *19*, 181.
[47] Miljkovic, Dj.; Cvetkovic, I.; Stosic-Grujicic, S.; Trajkovic, V. *Clin. Exp. Immunol.*, **2003,** *132*, 239.
[48] Nagai, M.; Natsumeda, Y.; Weber, G. *Cancer, Res.*, **1992,** *52*, 258.
[49] Paul, A.; Cuenda, A.; Bryant, C.E.; Murray, J.; Chilvers, E.R.; Cohen, P.; Gould, G.W.; Plevin, R. *Cell. Signal.*, **1999,** *11*, 491.
[50] Raab, M; Daxecker, H.; Karimi, A.; Markovic, S.; Cichna, M.; Markl, P.; Muller, M.M. *Clin. Chim. Acta.*, **2001,** *310*, 89.
[51] Saura, M.; Zaragoza, C.; Bao, C.; McMillan, A.; Lowenstein, C.J. *J. Mol. Biol.*, **1999,** *289*, 459.
[52] Schiff, M. *Am. J. Med.* **1997**, *102*, 11.
[53] Senda, M.; Delustro, B.; Eugui, E.; Natsumeda, Y. *Transplantation*, **1995,** *60*, 1143.
[54] Taniguchi, T.; Ogasawara, K.; Takaoka, A.; Tanaka, N. *Annu. Rev. Immunol.*, **2001,** *19*, 623.
[55] Tran, G.T.; Carter, N.; Hodgkinson, S.J. *Int. Immunopharmacol.*, **2001**, *1*, 1709.
[56] Vallee, S.; Fouchier, F.; Brauger, D.; Marvaldi, J.; Champion, S. *Eur. J. Pharmacol.*, **2000,** *404*, 49.
[57] Varley, C.L.; Dickson, A.J. *Biochem. Biophys. Res. Commun.*, **1999,** *263*, 627.

[58] Wang, Z.; Brecher, P. *Hypertension*, **1999,** *34*, 1259.
[59] Weigel, G.; Bertalanffy, P.; Dubsky, P.; Griesmacher, A.; Wolner, E. *Clin. Chem. Lab. Med.*, **1999,** *37*, 253.
[60] Weinberg, J.B. *Environ. Health. Perspect.*, **1998,** *106*, 1131.
[61] Willenborg, D.O.; Fordham, S.A.; Staykova, M.A.; Ramshaw, I.A.; Cowden, W.B. *J. Immunol.*, **1999**, *163*, 5278.
[62] Willenborg, D.O.; Staykova, M.A.; Cowden, W.B. *J. Neuroimmunol.* **1999**, *100*, 21.
[63] Wu, C.C.; Liao, M.H.; Chen, S.J.; Chou, T.C.; Chen, A.; Yen, M.H. *Shock*, **2000**, *14*, 60.
[64] Yu, C.C.; Yang, C.W.; Wu, M.S.; Ko, Y.C.; Huang, C.T.; Hong, J.J.; Huang, C.C. *J. Lab. Clin. Med.*, **2001,** *138*, 69.