adenovirus vector by an improved in vitro ligation method. Hum Gene Ther 1998;9:2577–83.
16. Tamamori-Adachi M, Ito H, Nobori K, Hayashida K, Kawauchi J, Adachi S, et al. Expression of cyclin D1 and CDK4 causes hypertrophic growth of cardiomyocytes in culture: a possible implication for cardiac hypertrophy. Biochem Biophys Res Commun 2002;296:274–80.
17. Tamamori-Adachi M, Ito H, Sumrejkanchanakij P, Adachi S, Hiroe M, Shimizu M, et al. Critical role of cyclin D1 nuclear import in cardiomyocyte proliferation. Circ Res 2003;92:e12–9.
18. Chang MW, Barr E, Seltzer J, Jiang YQ, Nabel GJ, Nabel EG, et al. Cytostatic gene therapy for vascular proliferative disorders with a constitutively active form of the retinoblastoma gene product. Science 1995;267:518–22.
19. Terao R, Honda K, Hatano E, Uehara T, Yamamoto M, Yamaoka Y. Suppression of proliferative cholangitis in a rat model with direct adenovirus-mediated retinoblastoma gene transfer to the biliary tract. Hepatology 1998;28:605–12.
20. Mizuguchi H, Koizumi N, Hosono T, Utoguchi N, Watanabe Y, Kay MA, et al. A simplified system for constructing recombinant adenoviral vectors containing heterologous peptides in the HI loop of their fiber knob. Gene Ther 2001;8:730–5.
21. Inoue H, Takamori M, Nagata N, Nishikawa T, Oda H, Yamamoto S, et al. An investigation of cell proliferation and soluble mediators induced by interleukin 1β in human synovial fibroblasts: comparative response in osteoarthritis and rheumatoid arthritis. Inflamm Res 2001;50:65–72.
22. Elshaw SR, Henderson N, Knox AJ, Watson SA, Buttle DJ, Johnson SR. Matrix metalloproteinase expression and activity in human airway smooth muscle cells. Br J Pharmacol 2004;142: 1318–24.
23. Pierer M, Rethage J, Seibl R, Lauener R, Brentano F, Wagner U, et al. Chemokine secretion of rheumatoid arthritis synovial fibroblasts stimulated by Toll-like receptor 2 ligands. J Immunol 2004;172:1256–65.
24. Zhong H, Simons JW. Direct comparison of GAPDH, β-actin, cyclophilin, and 28S rRNA as internal standards for quantifying RNA levels under hypoxia. Biochem Biophys Res Commun 1999; 259:523–26.
25. Zhu G, Conner SE, Zhou X, Shih C, Li T, Anderson BD, et al. Synthesis, structure-activity relationship, and biological studies of indolocarbazoles as potent cyclin D1-CDK4 inhibitors. J Med Chem 2003;46:2027–30.
26. Patel R, Bartosch B, Blank JL. $p21^{WAF1}$ is dynamically associated with JNK in human T-lymphocytes during cell cycle progression. J Cell Sci 1998;111:2247–55.
27. Bradley K, Scatizzi JC, Fiore S, Shamiyeh E, Koch AE, Firestein GS, et al. Retinoblastoma suppression of matrix metalloproteinase 1, but not interleukin-6, through a p38-dependent pathway in rheumatoid arthritis synovial fibroblasts. Arthritis Rheum 2004;50: 78–87.
28. Okada Y. Proteinases and matrix degradation. In: Ruddy S, Harris ED Jr, Sledge CB, Budd RC, Sergent JS, editors. Kelley's textbook of rheumatology. 6th ed. Philadelphia: WB Saunders; 2001. p. 55–72.
29. Tolboom TC, Pieterman E, van der Laan WH, Toes RE, Huidekoper AL, Nelissen RG, et al. Invasive properties of fibroblast-like synoviocytes: correlation with growth characteristics and expression of MMP-1, MMP-3, and MMP-10. Ann Rheum Dis 2002;61: 975–80.
30. Nagase H. Activation mechanisms of matrix metalloproteinases. Biol Chem 1997;378:151–60.
31. Van Meurs J, van Lent P, Stoop R, Holthuysen A, Singer I, Bayne E, et al. Cleavage of aggrecan at the Asn^{341}-Phe^{342} site coincides with the initiation of collagen damage in murine antigen-induced arthritis: a pivotal role for stromelysin 1 in matrix metalloproteinase activity. Arthritis Rheum 1999;42:2074–84.
32. Hamada T, Arima N, Shindo M, Sugama K, Sasaguri Y, Brewster M, et al. Suppression of adjuvant arthritis of rats by a novel matrix metalloproteinase-inhibitor Ro 32-3555, an orally active collagenase selective inhibitor, prevents structural damage in the STR/ORT mouse model of osteoarthritis. Ro 32-3555, an orally active collagenase inhibitor, prevents cartilage breakdown in vitro and in vivo. Br J Pharmacol 2000;131:1513–20.
33. Ishikawa T, Nishigaki F, Miyata S, Hirayama Y, Minoura K, Imanishi J, et al. Prevention of progressive joint destruction in adjuvant induced arthritis in rats by a novel matrix metalloproteinase inhibitor, FR217840: suppression of adjuvant arthritis of rats by a novel matrix metalloproteinase-inhibitor. Eur J Pharmacol 2005;508:239–47.
34. Lewis EJ, Bishop J, Bottomley KM, Bradshaw D, Brewster M, Broadhurst MJ, et al. Ro 32-3555, an orally active collagenase inhibitor, prevents cartilage breakdown in vitro and in vivo. Br J Pharmacol 1997;121:540–6.
35. Koch AE, Kunkel SL, Harlow LA, Johnson B, Evanoff HL, Haines GK, et al. Enhanced production of monocyte chemoattractant protein-1 in rheumatoid arthritis. J Clin Invest 1992;90:772–9.
36. Gong JH, Ratkay LG, Waterfield JD, Clark-Lewis I. An antagonist of monocyte chemoattractant protein 1 (MCP-1) inhibits arthritis in the MRL-lpr mouse model. J Exp Med 1997;186:131–7.
37. Wolff B, Naumann M. INK4 cell cycle inhibitors direct transcriptional inactivation of NF-κB. Oncogene 1999;18:2663–6.
38. Wasylyk C, Gutman A, Nicholson R, Wasylyk B. The c-Ets oncoprotein activates the stromelysin promoter through the same elements as several non-nuclear oncoproteins. EMBO J 1991;10: 1127–34.
39. White LA, Maute C, Brinckerhoff CE. ETS sites in the promoters of the matrix metalloproteinases collagenase (MMP-1) and stromelysin (MMP-3) are auxiliary elements that regulate basal and phorbol-induced transcription. Connect Tissue Res 1997;36: 321–35.
40. Senderowicz AM. Small-molecule cyclin-dependent kinase modulators. Oncogene 2003;22:6609–20.

Lipopolysaccharide-Induced Up-Regulation of Triggering Receptor Expressed on Myeloid Cells-1 Expression on Macrophages Is Regulated by Endogenous Prostaglandin E_2[1]

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Triggering receptor expressed on myeloid cells-1 (TREM-1) is a recently identified cell surface molecule that is expressed by neutrophils and monocytes. TREM-1 expression is modulated by various ligands for TLRs in vitro and in vivo. However, the influence of PGE_2, a potential mediator of inflammation, on TREM-1 expression has not been elucidated. In this study, we examined the effects of PGE_2 on LPS-induced TREM-1 expression by resident murine peritoneal macrophages (RPM) and human PBMC. PGE_2 significantly induced murine TREM-1 (mTREM-1) expression by RPM. Up-regulation of TREM-1 expression was specific to PGE_2 among arachidonic acid metabolites, while ligands for chemoattractant receptor-homologous molecule expressed on Th2 cells and the thomboxane-like prostanoid receptor failed to induce mTREM-1 expression. PGE_2 also increased expression of the soluble form of TREM-1 by PBMC. LPS-induced TREM-1 expression was regulated by endogenous PGE_2 especially in late phase (>2 h after stimulation), because cyclooxygenase-1 and -2 inhibitors abolished this effect at that points. A synthetic EP4 agonist and 8-Br-cAMP also enhanced mTREM-1 expression by RPM. Furthermore, protein kinase A, PI3K, and p38 MAPK inhibitors prevented PGE_2-induced mTREM-1 expression by RPM. Activation of TREM-1 expressed on PGE_2-pretreated PBMC by an agonistic TREM-1 mAb significantly enhanced the production of IL-8 and TNF-α. These findings indicate that LPS-induced TREM-1 expression on macrophages is mediated, at least partly, by endogenous PGE_2 followed by EP4 and cAMP, protein kinase A, p38 MAPK, and PI3K-mediated signaling. Regulation of TREM-1 and the soluble form of TREM-1 expression by PGE_2 may modulate the inflammatory response to microbial pathogens. *The Journal of Immunology,* **2007, 178: 1144–1150.**

Triggering receptor expressed on myeloid cells-1 (TREM-1)[3] is a recently discovered cell surface molecule that has been identified on neutrophils and monocytes (1, 2). The soluble form of TREM-1 (sTREM-1) is detected in bronchoalveolar lavage fluid from patients with microbial infection and has been demonstrated to act as an inhibitor of TREM-1 (3–6). TREM-1 is a 30-kDa glycoprotein belonging to the Ig superfamily and its expression is up-regulated by various ligands for TLRs (7–9). Activation of TREM-1 expressed on neutrophils and monocytes by an agonistic mAb has been shown to stimulate the expression of various proinflammatory cytokines, chemokines, and cell surface molecules (1, 7–9). Furthermore, LPS causes synergistic enhancement of cytokine production by monocytes in response to the agonistic mAb, indicating that TREM-1 amplifies inflammatory responses initiated by TLRs (1, 7–9). Although the natural ligands for TREM-1 have not been identified, its essential role in acute inflammatory responses has been demonstrated in murine models of septic shock, because blocking of TREM-1 by a sTREM-1 improves the survival of mice with bacterial sepsis (6, 9). Thus, activation of TREM-1 may play a crucial role in the inflammatory response to microbes.

PGs are multipotent mediators that modulate a number of pathophysiological responses. PGs are produced by metabolism of arachidonic acid through activation of cyclooxygenase (COX). COX has two isoforms, which are COX-1 and COX-2 (10). COX-1 is constitutively expressed, whereas COX-2 is expressed at low level by most normal resting cells. COX-2 expression is induced by various TLR ligands (11, 12) and release of PGs is significantly increased in various animal models of endotoxemia or sepsis (13, 14). In particular, PGE_2 has been shown to function as a mediator of sepsis-induced immunosuppression, an inhibitor of proinflammatory cytokine production by macrophages, and an inducer of IL-10 production (15). In contrast, PGE_2 has several detrimental effects in sepsis, including vasodilation and increased vascular permeability (16). Several previous studies have shown that COX inhibitors can improve the survival of mice after burn infection or administration of a lethal dose of LPS (17–19). These findings indicate that PGs play an important role in microbial inflammation, including sepsis or endotoxemia. However, the precise mechanisms by which PGs (particularly PGE_2) have a regulatory effect on microbial inflammation have not been determined.

Although TREM-1 is clearly induced by LPS, little is known regarding the biological influence of PGE_2 on TREM-1 during

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[3] Abbreviations used in this paper: TREM, triggering receptor expressed on myeloid cells; mTREM, murine TREM; hTREM, human TREM; sTREM, soluble form of TREM; hsTREM, human sTREM; PKA, protein kinase A; COX, cyclooxygenase; RPM, resident peritoneal macrophage; I-BOP, 1*S*-[1α,2α(*Z*),3β(1*E*,3*S*),4α]-7-[3-[3-hydroxy-4-(4-iodophenoxy)-1-butenyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid; TP, thromboxane-like prostanoid; CRTH2, chemoattractant receptor-homologous molecule expressed on Th2 cells; EP, E-series of prostaglandin.

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Table I. *Oligonucleotide primers and probes used for real-time PCR*

Target Gene	Type[a]	Primer or Probe (5′–3′)	Description (mer)
mTREM-1	F	CCAGAAGGCTTGGCAGAGACT	22
	B	ACTTCCCCATGTGGACTTCACT	22
mGAPDH	F	TGCAGTGGCAAAGTGGAGATT	21
	B	ATTTGCCGTGAGTGGAGTCAT	21
hTREM-1	F	GCCTTGTGCCCACTCTATACCA	22
	B	TGGAGACATCGGCAGTTGAC	20
	P	(FAM)CAGAACTGTGACCCAAGCTCCACCCA(TAMRA)	26
hsTREM-1	F	CCTCCCAAGGAGCCTCACA	19
	B	ACACCGGAACCCCTTGGT	18
	P	(FAM)CTGTTCGATCGCATCCGCTTGGT(TAMRA)	23

[a] F, forward primer; B, backward primer; and P, TaqMan Probe.

microbial inflammation. Therefore, we conducted this study to investigate the biological effects of PGE_2 on the expression and action of TREM-1.

Materials and Methods

Reagents

DI-004 (an EP1 agonist), AE1–259-01 (an EP2 agonist), AE-248 (an EP3 agonist), and AE1-329 (an EP4 agonist) were provided by Ono Pharmaceuticals. A monoclonal rat anti-mouse TREM-1 Ab and a monoclonal mouse anti-human TREM-1 Ab, as well as control mouse IgG1 and a polyclonal anti-actin Ab, were obtained from R&D Systems and Santa Cruz Biotechnology, respectively. HRP-conjugated rabbit anti-mouse IgG and HRP-conjugated rabbit anti-rat IgG were purchased from DakoCytomation. Specific ELISAs for human TNF-α and human IL-8 were obtained from BioSource International. 8-Bromoadenosine 3′, 5′ cyclic monophosphate (8-Br-cAMP), LPS, the MEK (MAPKK) inhibitor PD98059, the p38 MAPK inhibitor SB203580, and the PI3K inhibitor LY294002 were purchased from Sigma-Aldrich, while the protein kinase A (PKA) inhibitor H-89 was obtained from Seikagaku. PGD_2, PGE_2, 1*S*-[1α, 2α(*Z*),3β(1*E*,3*S*),4α]-7-[3-[3-hydroxy-4-(4-iodophenoxy)-1-butenyl]-7-oxabicyclo[2,2,1]hept-2-yl]-5-heptenoic acid (I-BOP), a COX-1 inhibitor (SC560), a COX-2 inhibitor (NS398), and a PGE_2 EIA kit were obtained from Cayman Chemical.

Cell culture

Resident peritoneal macrophages (RPM) were isolated from male ICR mice (6–8 wk old) as reported elsewhere (15). Heparinized peripheral blood was obtained from healthy volunteers and human PBMC were isolated by density-gradient centrifugation with Ficoll-Paque. After washing with PBS, the RPM or PBMC were suspended in RPMI 1640 medium (Sigma-Aldrich) supplemented with 5% heat-inactivated FCS (HyClone), 100 U/ml penicillin, and 100 μg/ml streptomycin (Invitrogen Life Technologies) for culture under a 5% CO_2 atmosphere at 37°C.

RPM (1×10^6 cells) or PBMC (2×10^6 cells) were incubated for the indicated periods with or without various concentrations of PGs (PGD_2, PGE_2, I-BOP), 8-Br-cAMP, LPS, or E-series of prostaglandin (EP EP1–4) agonists. Then the expression of murine TREM-1 (mTREM-1), human TREM-1 (hTREM-1), and soluble hTREM-1 (hsTREM-1) was investigated.

RPM were incubated in the presence or absence of SC560 and/or NS398 for 1 h to inhibit endogenous COX activity, and then the cells were incubated in the presence of LPS for the indicated periods. To block protein kinase activity, RPM were incubated in the presence or absence of various inhibitors such as SB203580, PD98059, LY294002, or H89 for 30 min, after which the cells were incubated with LPS or PGE_2 for the indicated periods.

At the termination of incubation, cells and culture supernatants were obtained by centrifugation. Total RNA and protein were isolated by using RLT lysis buffer (Qiagen). Samples of cell lysate and culture medium were stored at −80°C until use.

Quantitative real-time PCR

Total RNA was extracted from cell lysates using an RNeasy Mini kit (Qiagen). The RNA was treated with DNase I (Qiagen) and cDNA was synthesized from 2 μg of random-primed total RNA in a volume of 20 μl using Omniscript reverse transcriptase (Qiagen). mTREM-1, GAPDH, and COX-2 were assessed by quantitative real-time PCR (SYBR) using specific oligonucleotide primers. hTREM-1, hsTREM-1, and rRNA were assessed by quantitative real-time PCR (TaqMan) using specific oligonucleotide primers and probe. hsTREM-1 was identified as a splice variant of hTREM-1 with a 193-base deletion (exon 3) from bases 471 to 663 (GenBank accession no. AF287008). To avoid amplification of hsTREM-1 mRNA, the forward primer for hTREM-1 was designed to fit exon 3 (the deletion site). To amplify only hsTREM-1, the backward primer was designed to hybridize to the 3′ end of exon 2 as well as the 5′ end of exon 4. These primers could specifically amplify hTREM-1 and hsTREM-1, respectively. The sequences of the primers and probes are listed in Table I. The rRNA primers and probe were purchased from Applied Biosystems. The SYBR PCR was performed in duplicate using a 25-μl reaction mixture containing 1 μl of cDNA, QuantiTect SYBR Green PCR (Qiagen), and 300 nM each of the sense and antisense primers. The PCR mixture was incubated for 15 min at 95°C, and then amplification was performed for 45 cycles, consisting of denaturation at 94°C for 15 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s. TaqMan PCR was performed in duplicate with a 25-μl reaction mixture containing 1 μl of cDNA, 12.5 μl of QuantiTect Probe PCR (Qiagen), 400 nM each of the sense and antisense primers, and 200 nM of the probe. The PCR mixture was incubated for 15 min at 95°C to activate HotStarTaq DNA polymerase. Subsequently, amplification was performed for 45 cycles, consisting of denaturation at 94°C for 15 s and combined annealing extension at 59°C for 1 min. During the extension step, the ABI Prism 7700 Sequence Detection System monitored PCR amplification in real time by quantitative analysis of the emitted fluorescence. The amount of each sample mRNA was evaluated relative to the control sample, which was assigned a value of 1 arbitrary unit.

Western blot analysis

Culture medium or RPM (1×10^6 cells) was dissolved in sample buffer (350 mM Tris (pH 6.8), 10% SDS, 30% glycerol, 600 mM DTT, and 0.05% bromphenol blue), loaded onto 10% SDS-PAGE gel, and run at 20 mA for 1.5 h. Proteins in the supernatant were transferred to a polyvinylidene difluoride membrane (Roche Diagnostics) for 1.5 h at 200 mA by semidry blotting. The membrane was then blocked with 5% skim milk in PBS containing 0.05% Tween 20 for 1 h at 37°C, washed with PBS containing 0.1% Tween 20, and incubated overnight at 4°C with a monoclonal anti-human TREM-1 Ab (1 μg/ml). The blots were washed four times with TBS and incubated for 30 min with HRP-conjugated rabbit anti-mouse IgG. Immunoreactive bands were developed using a chemiluminescent substrate (ECL plus; Amersham Biosciences).

Assay of cytokine and chemokine production

Flat-bottom plates were precoated with 5 μg/ml of a monoclonal anti-human TREM-1 Ab or an isotype-matched control Ab (mouse IgG1) overnight at 4°C. After washing with PBS, PBMC (1×10^5 cells) were preincubated with or without PGE_2 (1 μM) for 5 h. Then the PBMC were added to the Ab-coated wells, and briefly spun in a centrifuge at 1200 rpm to bind TREM-1. After incubation for 24 h, culture medium was obtained by centrifugation and stored at −20°C until the levels of TNF-α and IL-8 in the supernatant were determined by specific ELISAs.

Assay of PGE_2 production

Concentration of PGE_2 in the culture supernatant was determined by using a PGE_2 EIA kit according to the manufacturer's instructions.

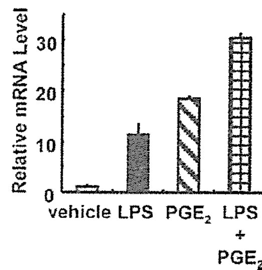

FIGURE 1. PGE_2 and LPS up-regulate mTREM-1 expression by RPM. RPM were incubated with or without PGE_2 (1 μM) for 1 h, and then were cultured in the presence or absence of LPS (100 ng/ml). The mTREM-1 mRNA level was determined by quantitative real-time PCR using murine GAPDH as the internal control. The relative level of mTREM-1 mRNA was evaluated by comparison with that in vehicle (EtOH)-treated RPM, which was defined as 1 arbitrary unit. Data are expressed as the mean ± SD of triplicate determinations.

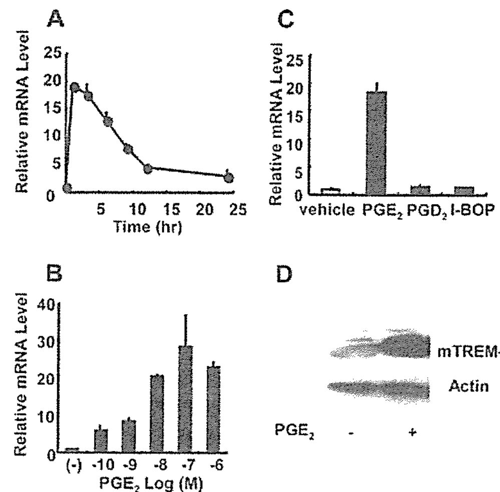

FIGURE 2. PGE_2 induces mTREM-1 expression in a time- or concentration-dependent manner. *A*, RPM were incubated with PGE_2 (1 μM) for the indicated periods and the mTREM-1 mRNA level was determined by quantitative real-time PCR. *B*, RPM were cultured with or without various concentrations of PGE_2 for 1 h and the mTREM-1 mRNA level was determined by quantitative real-time PCR. *C*, RPM were incubated with PGE_2 (1 μM), PGD_2 (1 μM), or I-BOP (0.2 μM) for 1 h and the mTREM-1 mRNA level was determined by quantitative real-time PCR. *D*, RPM were cultured with or without PGE_2 (1 μM) for 5 h, and expression of mTREM-1 protein and actin was determined by Western blot analysis. Data are expressed as the mean ± SD of triplicate determinations.

Statistical analysis

Results are expressed as the mean ± SD. Statistical analysis was performed using the paired Student *t* test and $p < 0.05$ was considered to indicate significance.

Results

PGE_2 induces TREM-1 expression by RPM

PGE_2 is a mediator with a wide variety of biological effects in the process of microbial inflammation. To determine whether PGE_2 could influence the expression and action of TREM-1 in macrophages, RPM were pretreated with PGE_2 at a concentration of 1 μM for 1 h and then the cells were subsequently incubated in the presence or absence of LPS (100 ng/ml) for 1 h. Expression of mTREM-1 was determined by quantitative real-time PCR. LPS significantly increased expression of the *TREM-1* gene (Fig. 1), as previously reported. PGE_2 also caused significant induction of TREM-1 expression and the magnitude of gene expression was significantly higher in PGE_2-treated cells than in LPS-treated cells. Furthermore, a combination of PGE_2 and LPS caused additive enhancement of mTREM-1 expression by RPM.

To investigate the time course of PGE_2-induced expression of mTREM-1, RPM were incubated with 1 μM PGE_2 for the indicated periods. Induction of gene expression occurred quite rapidly and was observed as early as 1 h after stimulation, following declined for 12 h (Fig. 2*A*). RPM were incubated with varying concentrations of PGE_2 for 1 h to determine whether physiological levels of PGE_2 enhanced mTREM-1 expression. It was shown that PGE_2 increased mTREM-1 expression in a concentration-dependent manner. PGE_2 at a concentration as low as 10^{-10} M significantly induced mTREM-1 expression and maximal expression occurred after stimulation with 10^{-6}–10^{-7} M PGE_2 (Fig. 2*B*).

It has been demonstrated that monocytes and macrophages express various receptors for arachidonic acid metabolites, which are referred to EP, thromboxane-like prostanoid (TP), and CRTH2 (20). Therefore, we investigated the effects of specific ligands for these receptors on mTREM-1 expression by RPM. The cells were incubated for 1 h with PGE_2 (an EP receptor ligand), I-BOP (a TP receptor ligand), or PGD_2 (a CRTH2 ligand), and TREM-1 expression was evaluated by quantitative real-time PCR. PGE_2 induced TREM-1 expression, while neither I-BOP nor PGD_2 up-regulated mTREM-1 expression, indicating that PGE_2 was a specific inducer of mTREM-1 expression among these PGs (Fig. 2*C*).

Western blot analysis using a specific anti-mouse TREM-1 mAb was performed to evaluate mTREM-1 protein expression by RPM after incubation with or without PGE_2 for 5 h. mTREM-1 protein was detected faintly when RPM were incubated with the vehicle alone, whereas increased expression of mTREM-1 was clearly seen when RPM were incubated with PGE_2 (10^{-6} M) for 5 h (Fig. 2*D*).

Endogenous PGE_2 induces TREM-1 expression by RPM

It has been demonstrated that LPS induces TREM-1 expression as well as the release of PGE_2 by macrophages (7–9, 21). To evaluate the possible influence of endogenous PGE_2 on LPS-induced mTREM-1 expression, RPM were stimulated with LPS (100 ng/ml) for the indicated periods, after which PGE_2 production and *mTREM-1* gene expression were determined by EIA and quantitative real-time PCR, respectively. PGE_2 synthesis gradually increased up to 4 h, and then the maximum level was maintained

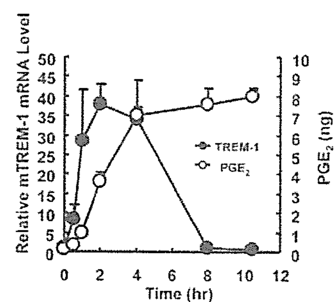

FIGURE 3. LPS-induced PGE_2 production up-regulates mTREM-1 expression by RPM. RPM were stimulated with LPS (100 ng/ml) for the indicated periods. Then the mTREM-1 mRNA level was determined by quantitative real-time PCR, and PGE_2 synthesis was determined by using a PGE_2 EIA kit. Data are expressed as the mean ± SD of triplicate determinations.

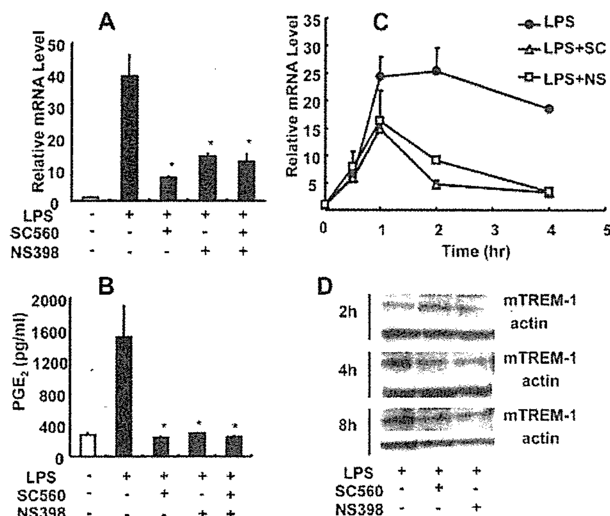

FIGURE 4. Effect of COX inhibitors on LPS-induced mTREM-1 expression by RPM. RPM were pretreated with or without SC560 and/or NS398 for 1 h, and were subsequently incubated in the presence or absence of LPS (100 ng/ml) for the indicated periods. *A*, The mTREM-1 mRNA level at 2 h after LPS stimulation was determined by quantitative real-time PCR. *B*, PGE_2 synthesis at 2 h after LPS stimulation was determined by a PGE_2 EIA kit. *C*, Time course of mTREM-1 mRNA expression was determined by quantitative real-time PCR. *D*, mTREM-1 protein and actin was determined by Western blot analysis. Data are expressed as the mean ± SD of triplicate determinations. *, $p < 0.01$, vs LPS-stimulated RPM by Student's unpaired *t* test.

until 11 h after stimulation (Fig. 3). In contrast, gene expression of mTREM-1 occurred quite rapidly and was seen as early as 0.5 h after stimulation when PGE_2 production was not detected. Maximum induction of mTREM-1 was observed at 2–4 h after stimulation and gene expression returned to the basal level by 8 h. These findings indicated that PGE_2 synthesis did not precede the induction of *mTREM-1* gene expression.

mTREM-1 expression was significantly induced by a physiological concentration of PGE_2. Therefore, the regulatory roles of PGE_2 on TREM-1 expression was investigated. Because PGE_2 synthesis is regulated by COX-1 and COX-2, RPM were incubated for 1 h in the presence or absence of SC560 (a selective COX-1 inhibitor) or NS398 (a selective COX-2 inhibitor), and then the cells were stimulated with LPS for 2 h. mTREM-1 expression and PGE_2 synthesis was determined by quantitative real-time PCR and EIA, respectively. Both inhibitors for COX-1 and COX-2 partially, but significantly, inhibited LPS-induced expression of mTREM-1 (Fig. 4*A*). When the effects of COX inhibitors on PGE_2 synthesis by LPS-stimulated RPM were investigated, these inhibitors also suppressed PGE_2 synthesis (Fig. 4*B*). Vehicle (DMSO) did not affect on LPS-induced PGE_2 synthesis and mTREM-1 expression (data not shown).

To investigate the effect of PGE_2 on LPS-induced mTREM-1 mRNA expression at early time points, RPM were stimulated with LPS in the presence or absence of COX inhibitors for the indicated periods. Both COX-1 and COX-2 inhibitors failed to inhibit mTREM-1 expression at 0.5 h after LPS stimulation, whereas mTREM-1 expression at 1 h was partially inhibited, and that at 2 and 4 h was significantly abolished by COX inhibitors (Fig. 4*C*). These findings indicated that the effect of PGE_2 on LPS-induced mTREM-1 expression was predominant at late time points (>2 h after stimulation) but not at early time points (0.5 and 1 h after stimulation).

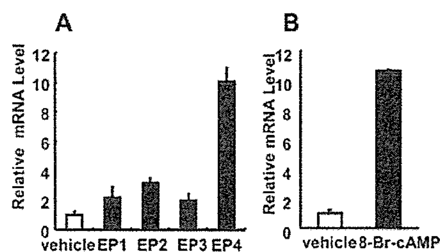

FIGURE 5. Enhancement of mTREM-1 expression by an EP4 agonist and cAMP analog. *A*, RPM were incubated with or without agonists for EP1 to EP4 agonists (1 μM) for 1 h and the mTREM-1 mRNA level was determined by quantitative real-time PCR. *B*, RPM were incubated with or without 8-Br-cAMP (0.5 mM) for 1 h. Data are expressed as the mean ± SD of triplicate determinations.

After RPM were incubated with LPS in the presence or absence of COX inhibitors for various times, the expression of mTREM-1 protein was determined by Western blot analysis. Both inhibitors reduced LPS-induced TREM-1 expression at 4 and 8 h but not at 2 h after stimulation (Fig. 4*D*). Actin as the internal control was similarly detected in all samples. These findings indicated that LPS-induced expression of mTREM-1 on RPM was at least partly promoted by endogenous PGE_2.

PGE_2-induced TREM-1 expression is mediated by the EP4 receptor and cAMP

It has been shown that the biological functions of PGE_2 are mediated by four specific receptors, which are coupled to G-protein and are referred to as EP1 to EP4 (20). To determine which EP receptors mediated PGE_2-induced mTREM-1 expression, RPM were incubated with four synthetic agonists specific for each of the EP receptors (each at a concentration of 1 μM), and then mTREM-1 expression was evaluated by quantitative real-time PCR. The EP4 agonist significantly up-regulated TREM-1 expression in RPM, whereas the EP1, EP2, and EP3 agonists failed to enhance TREM-1 expression (Fig. 5*A*).

Activation of the EP4 receptor enhances intracellular accumulation of cAMP via adenylate cyclase. Therefore, we examined the influence of 8-Br-cAMP (a stable cAMP analog) on mTREM-1 expression by RPM. Treatment of RPM with 8-Br-cAMP at a concentration of 5×10^{-4} M for 1 h significantly enhanced expression of the *mTREM-1* gene by RPM (Fig. 5*B*). EP4 agonist and 8-Br-cAMP also induced TREM-1 mRNA expression in J774.1 and PBMC (data not shown). These finding clearly suggested that PGE_2-induced TREM-1 expression on RPM was related to EP4 receptor- and cAMP-mediated signaling.

Blocking of PKA, p38 MAPK, and PI3K inhibits PGE_2-induced mTREM-1 expression

Intracellular cAMP is a major regulator of PKA (22) and cAMP also activates the PI3K-, p38 MAPK-, and ERK-signaling pathways (23–25). Therefore, we investigated the signaling pathways involved in PGE_2-induced expression of TREM-1 by using synthetic inhibitors of these kinases. A PKA inhibitor (H89), a p38 MAPK inhibitor (SB203580), and a PI3K inhibitor (LY294002) significantly suppressed PGE_2-induced TREM-1 expression, whereas a MAPKK inhibitor (PD98059) failed to influence TREM-1 expression (Fig. 6). Inhibitory effects of these inhibitors were observed in a dose- or time-dependent manner (data not shown). These results suggested that PGE_2-induced TREM-1 expression was mediated via the PKA, PI3K, and p38 MAPK pathways.

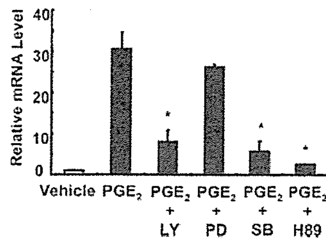

FIGURE 6. Inhibition of PKA, p38 MAPK, or PI3K suppresses PGE_2-induced mTREM-1 expression by RPM. RPM were pretreated with or without LY294002 (20 μM), PD98059 (30 μM), SB203580 (20 μM) H-89 (20 μM) for 30 min, and then were incubated with PGE_2 (1 μM) for 1 h. The mTREM-1 mRNA level was determined by quantitative real-time PCR. Data are expressed as the mean ± SD of triplicate determinations. *, $p < 0.01$ vs PGE_2-stimulated RPM by Student's unpaired t test.

Activation of TREM-1 significantly enhances cytokine production by PGE_2-treated PBMC

An agonistic anti-TREM-1 mAb has been shown to stimulate the production of proinflammatory cytokines by monocytes (1, 7, 9). It was difficult to transfer PGE_2-treated RPM to Ab-coated wells, because the cells tightly adhere to the culture dishes. Therefore, PBMC were used to determine whether TREM-1 could enhance cytokine production by PGE_2-treated monocytes. Cells were incubated in the presence or absence of PGE_2 (10^{-6} M) for 5 h, and then harvested for incubation in agonistic anti-TREM-1 mAb-coated wells for 24 h. Then the levels of TNF-α and IL-8 in the culture supernatant were determined by specific ELISAs. The agonistic anti-TREM-1 mAb caused a significant increase of TNF-α production by PGE_2-pretreated PBMC (Fig. 7*A*). Production of TNF-α by PGE_2-treated cells was 6-fold higher than that by untreated cells. The agonistic anti-TREM-1 mAb also increased IL-8 production by PGE_2-pretreated PBMC and the magnitude of this enhancement was 4.6-fold (Fig. 7*B*). These results indicated that

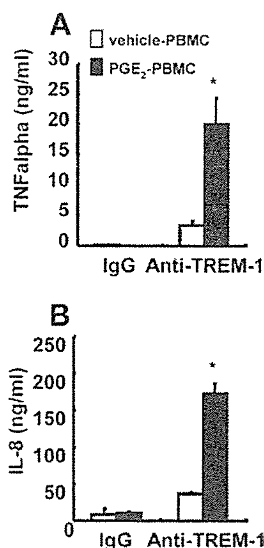

FIGURE 7. An agonistic anti-TREM-1 mAb enhances the production of proinflammatory cytokines by PGE_2-pretreated PBMC. PBMC were incubated with or without PGE_2 for 5 h, and then the cells were incubated in the presence or absence of the agonistic anti-TREM-1 mAb (5 μg/ml) or an isotype control Ab (5 μg/ml) for 24 h. Production of TNF-α (*A*) and IL-8 (*B*) was determined by specific ELISAs. Data are expressed as the mean ± SD of triplicate determinations. *, $p < 0.01$ vs vehicle-stimulated PBMC by Student's unpaired t test.

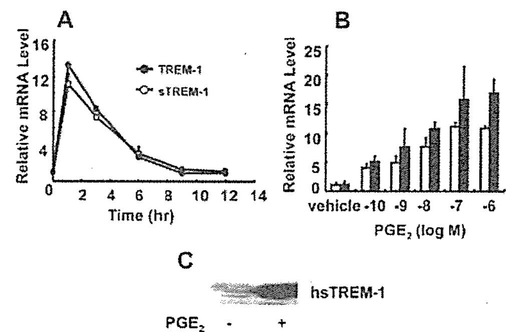

FIGURE 8. PGE_2 induces hTREM-1 and hsTREM-1 expression by PBMC in a time- and concentration-dependent manner. *A*, PBMC were incubated with PGE_2 (1 μM) for the indicated periods. Expression of the *hTREM-1* gene (○) and the *hsTREM-1* gene (●) was determined by real-time quantitative PCR. *B*, PBMC were cultured with or without various concentrations of PGE_2 for 1 h. Then the hTREM-1 (□) and hsTREM-1 (■) mRNA levels were determined by real-time quantitative PCR. *C*, PBMC were incubated with or without PGE_2 (1 μM) for 5 h and hsTREM-1 protein in the same volume of the supernatant (20μl) was determined by Western blot analysis. Data are expressed as the mean ± SD of triplicate determinations.

TREM-1 induced by PGE_2 was functional and enhanced the production of proinflammatory cytokines by PBMC.

PGE_2 induces hTREM-1 and hsTREM-1 expression by PBMC

It has been demonstrated that human monocytes are capable of expressing sTREM-1 as well as the cell surface form (3–5). Although sTREM-1 has also been identified in mice, the precise structure and function of soluble mTREM-1 are not yet known. Therefore, we investigated the expression of hTREM-1 and hsTREM-1 by PGE_2-treated PBMC to evaluate which type was predominantly expressed. After PBMC were incubated with PGE_2, *hTREM-1* and *hsTREM-1* gene expression were separately determined by quantitative real-time PCR.

Induction of both *hTREM-1* and *hsTRM-1* gene expression occurred quite rapidly and was seen as early as 1 h after stimulation, a subsequent declined until 9 h (Fig. 8*A*). To determine whether physiological concentrations of PGE_2 could induce the expression of hTREM-1 and hsTREM-1, PBMC were incubated with various concentrations of PGE_2 for 1 h and gene expression was determined. It was found that PGE_2 promoted both hTREM-1 and hsTREM-1 expression in a concentration-dependent manner, with maximal expression occurring at 10^{-6} or 10^{-7} M (Fig. 8*B*).

Western blot analysis using a specific anti-human TREM-1 mAb was performed to detect sTREM-1 protein. PBMC were incubated with or without PGE_2 for 5 h, and then the sTREM-1 protein level in the culture supernatant was determined by Western blotting. sTREM-1 was detected at very low levels when PBMC were incubated with the vehicle alone, whereas sTREM-1 expression was increased when PBMC were incubated with PGE_2 for 5 h (Fig. 8*C*). Taken together, these findings showed that PGE_2 up-regulated the expression of hTREM-1 as well as hsTREM-1 by monocytes.

Discussion

The present study provided evidence that PGE_2 up-regulates mTREM-1 expression by RPM, as well as hTREM-1 and hsTREM-1 expression by human PBMC. LPS-induced TREM-1 expression is at least partly regulated by endogenous PGE_2, because COX inhibitors significantly reduced TREM-1 expression. PGE_2-induced TREM-1 expression was mediated by EP-4, cAMP, and various kinases such as PKA, PI3K, and p38 MAPK. PGE_2-induced TREM-1 was functional,

because agonistic anti-TREM-1 mAb promoted a significant increase in the production of TNF-α and IL-8.

It is known that TREM-1 is specifically up-regulated by microbial products such as LPS, lipoteichoic acid (LTA), or zymosan (1, 7, 9, 26). However, the present study provided the first demonstration that PGE_2 could induce TREM-1 expression by both RPM and PBMC. Induction of TREM-1 expression by PGE_2 was also observed in a human monocyte cell line (U937) and a murine macrophage cell line (J774.1) (data not shown), indicating that PGE_2 is an inducer of TREM-1 expression by both monocytes and macrophages. PGE_2 was a specific regulator of mTREM-1 expression, because specific ligands for the CRTH2 and TP receptors (which are expressed on macrophages) failed to induce mTREM-1 expression. Biological effect of endogenous PGE_2 on TREM-1 expression was predominant in the late phase of LPS-induced TREM-1 expression. This is based on the findings that COX inhibitors abrogated mTREM-1 expression after 2 h and also reduced mTREM-1 protein expression after 4–8 h following LPS stimulation.

In the present study, both COX-1 and COX-2 inhibitors suppressed PGE_2 synthesis and TREM-1 induction. It has been documented that LPS promoted PGE_2 production through the induction and activation of the COX-2, but not COX-1 (21, 27). However, Rouzer et al. (21) also reported that SC560 (COX-1 inhibitor) inhibits PG synthesis through inhibition of COX-2 as well as COX-1 in LPS-stimulated RPM. Because this cross-inhibition was not observed in other cells, it appeared to be specific in RPM. TNF-α is also an inducer of PGE_2 synthesis, but a previous study demonstrated that TNF-α had a limited effect on TREM-1 expression (7, 28). The reasons for this difference are not known, but it might be related to different mechanisms of action on monocytes and macrophages.

EP4, one of the receptors for PGE_2, increases intracellular cAMP levels via activation of adenylate cyclase and promotes activation of the PKA, PI3K, p38 MAPK, and MAPKK pathways (22–25). The present study demonstrated that a specific EP4 agonist and 8-Br-cAMP both enhanced *mTREM-1* gene expression, while inhibitors of PKA, p38 MAPK, and PI3K blocked the PGE_2-induced increase of mTREM-1 expression. These findings suggested that PGE_2-induced up-regulation of mTREM-1 expression was mediated by the binding of PGE_2 to EP4, which was followed by accumulation of cAMP and activation of various kinases, including PKA, p38 MAPK, and PI3K. This is consistent with the findings of previous studies demonstrating that PGE_2 potentially activate various kinases such as PKA, PI3K, and p38 MAPK independently (29, 30). COX inhibitors failed to completely suppress LPS-induced up-regulation of mTREM-1 expression by RPM, and these inhibitors abolished TREM-1 expression only in the late phase, but not early phase of LPS stimulation. These indicate that other pathways might also be involved in the induction of TREM-1 expression by LPS. Knapp et al. (28) recently demonstrated that a PI3K-dependent pathway played a central role, while MAPK also played a limited role, in LPS-induced up-regulation of TREM-1 expression by monocytes. Several signaling pathways might be involved in LPS-induced TREM-1 expression, and the endogenous PGE_2-mediated pathway seems to be one of the mechanisms of LPS-induced TREM-1 expression on monocytes and macrophages.

TLR and TREM-1 cooperate to induce an inflammatory response, because activation of TREM-1 causes a marked increment in the production of proinflammatory cytokines by macrophages when LPS is used as the costimulus (1, 8, 9). TREM-1 activates a downstream signaling pathway through DAP12, which involves tyrosine phosphorylation, activation of mitogen-activated protein kinases, and mobilization of Ca^{2+}. In contrast, TLRs directly recognize certain microbial products and components, such as LPS, LTA, and bacterial DNA. MyD88, IRAK, TRAF6, and IKK are essentially involved in the TLR-signaling pathway. These kinases can potentially induce the production of proinflammatory cytokines via the activation of NF-κB (31). Natural ligands for TREM-1 remain to be identified. If specific ligands for TREM-1 are located at the foci of microbe-induced inflammation, interactions between TREM-1 and TLRs can synergistically induce inflammatory responses. In this case, cooperation between TLRs and TREM-1 could occur at several levels during the process of LPS-induced inflammation. The present study showed that an LPS-induced increase in the production of PGE_2 promoted TREM-1 expression, and activation of TREM-1 on PGE_2-treated PBMC enhanced the production of proinflammatory cytokines. Based on these findings, we hypothesized that PGE_2-induced up-regulation of TREM-1 expression may play an important role in enhancing the TLR-mediated response of macrophages to LPS stimulation.

Several line of evidence indicated that decoy receptors can modulate inflammatory responses by blocking the action by agonists (32, 33). sTREM-1 is a natural decoy receptor that could potentially inhibit TREM-1-mediated activation of cells through competition with natural ligand(s) for receptor binding. Synthetic sTREM-1 has been shown to inhibit LPS-induced cytokine production by monocytes in vitro (6). Furthermore, a recombinant sTREM-1 fusion protein and synthetic soluble TREM-1 have been shown to protect mice against lethal LPS challenge or bacterial sepsis by suppressing inflammatory cytokine production (6, 9). In contrast, it has been demonstrated that PGE_2 can suppress the production of various cytokines (such as TNF-α, IL-8, MCP-1, IFN-γ-inducible protein-10, and MIP-1β) by LPS-stimulated macrophages through EP2- and/or EP4-mediated pathways (34, 35). PGE_2 also induces the production of IL-10, which can have an anti-inflammatory effect (36). Present study demonstrated that PGE_2 induced the release of sTREM-1 by PBMC. Therefore, PGE_2 might suppress inflammation not only by inhibiting the production of proinflammatory cytokines, but also by inducing expression of the decoy receptor sTREM-1 and increasing the production of IL-10. However, activation of TREM-1 on PGE_2-treated PBMC enhanced the production of proinflammatory cytokines, indicating that PGE_2 may exert bidirectional effects on monocytes and macrophages to modulate inflammation through altering the expression of TREM-1 and sTREM-1.

Blocking of PGs has been shown to increase LPS-induced cytokine production both in vitro and in vivo (36–39). This is consistent with the previous finding that PGE_2 and EP4 agonists attenuated LPS-induced cytokine production in mice (40). However, a number of studies have provided evidence that COX inhibitors can improve survival after the onset of endotoxic shock and COX-deficient mice are resistant to endotoxin-induced inflammation and death (17, 19, 41). Thus, the precise pathophysiological role of PGE_2 in microbe infection still remains undefined. Further investigations should be directed toward the in vivo effects of PGE_2-induced TREM-1 and sTREM-1 in sepsis models.

Increased expression of TREM-1 has been observed at sites of inflammation caused by microbial pathogens (9). However, we recently demonstrated that monosodium urate monohydrate (MSU) crystals induced mTREM-1 expression in monocytes and macrophages in vitro and in vivo (42), indicating that TREM-1 might be involved in the development of acute gouty arthritis. We also observed that MSU crystal-induced mTREM-1 expression is regulated, at least in part, by endogenous produced PGE_2 (our unpublished data). These findings suggest the possibility that PGE_2 might enhance TREM-1 expression in nonmicrobial inflammatory diseases including acute gouty arthritis. If a natural TREM-1 ligand is also induced in nonmicrobial inflammation, it could enhance inflammatory responses by activating PGE_2-induced

TREM-1. Furthermore, nonmicrobial products such as heat shock protein 60, which are induced in various inflammatory diseases, have been shown to stimulate TLRs (43). Thus, it is presumed that activation of PGE_2-induced TREM-1 and TLRs by specific ligands might cooperatively increase the inflammatory responses in patients with nonmicrobial inflammatory diseases.

The present study provided a first evidence that PGE_2 induces the expression of both TREM-1 and sTREM-1 by macrophages. This finding sheds new light on the role of PGE_2 as a regulator of the inflammatory response to microbial infection. Further investigations should be directed toward the assessment of pathophysiological roles of TREM-1 and sTREM-1 in various inflammatory diseases. Such studies may help to elucidate the precise role of PGE_2-induced TREM-1 expression in inflammation and could possibly provide evidence leading to new strategies for the treatment of inflammatory diseases.

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Disclosures

The authors have no financial conflict of interest.

References

1. Bouchon, A., J. Dietrich, and M. Colonna. 2000. Inflammatory responses can be triggered by TREM-1, a novel receptor expressed on neutrophils and monocytes. *J. Immunol.* 164: 4991–4995.
2. Colonna, M., and F. Facchetti. 2003. TREM-1 (triggering receptor expressed on myeloid cells): a new player in acute inflammatory responses. *J. Infect. Dis.* 187: S397–S401.
3. Allaouchiche, B., and E. Boselli. 2004. Soluble TREM-1 and the diagnosis of pneumonia. *N. Engl. J. Med.* 350: 1904–1905.
4. Gibot, S., M. N. Kolopp-Sarda, M. C. Bene, A. Cravoisy, B. Levy, G. C. Faure, and P. E. Bollaert. 2004. Plasma level of a triggering receptor expressed on myeloid cells-1: its diagnostic accuracy in patients with suspected sepsis. *Ann. Intern. Med.* 141: 9–15.
5. Gingras, M. C., H. Lapillonne, and J. F. Margolin. 2002. TREM-1, MDL-1, and DAP12 expression is associated with a mature stage of myeloid development. *Mol. Immunol.* 38: 817–824.
6. Gibot, S., M. N. Kolopp-Sarda, M. C. Bene, P. E. Bollaert, A. Lozniewski, F. Mory, B. Levy, and G. C. Faure. 2004. A soluble form of the triggering receptor expressed on myeloid cells-1 modulates the inflammatory response in murine sepsis. *J. Exp. Med.* 200: 1419–1426.
7. Bleharski, J. R., V. Kiessler, C. Buonsanti, P. A. Sieling, S. Stenger, M. Colonna, and R. L. Modlin. 2003. A role for triggering receptor expressed on myeloid cells-1 in host defense during the early-induced and adaptive phases of the immune response. *J. Immunol.* 170: 3812–3818.
8. Colonna, M. 2003. TREMs in the immune system and beyond. *Nat. Rev. Immunol.* 3: 445–453.
9. Bouchon, A., F. Facchetti, M. A. Weigand, and M. Colonna. 2001. TREM-1 amplifies inflammation and is a crucial mediator of septic shock. *Nature* 410: 1103–1107.
10. Marnett, L. J., S. W. Rowlinson, D. C. Goodwin, A. S. Kalgutkar, and C. A. Lanzo. 1999. Arachidonic acid oxygenation by COX-1 and COX-2: mechanisms of catalysis and inhibition. *J. Biol. Chem.* 274: 22903–22906.
11. Bertelsen, L. S., G. Paesold, L. Eckmann, and K. E. Barrett. 2003. *Salmonella* infection induces a hypersecretory phenotype in human intestinal xenografts by inducing cyclooxygenase 2. *Infect. Immun.* 71: 2102–2109.
12. Lopez-Urrutia, L., A. Alonso, Y. Bayon, M. L. Nieto, A. Orduna, and M. Sanchez Crespo. 2001. *Brucella* lipopolysaccharides induce cyclooxygenase-2 expression in monocytic cells. *Biochem. Biophys. Res. Commun.* 289: 372–375.
13. Anderson, F. L., W. Jubiz, T. J. Tsagaris, and H. Kuida. 1975. Endotoxin-induced prostaglandin E and F release in dogs. *Am. J. Physiol.* 228: 410–414.
14. Kessler, E., R. C. Hughes, E. N. Bennett, and S. M. Nadela. 1973. Evidence for the presence of prostaglandin-like material in the plasma of dogs with endotoxin shock. *J. Lab. Clin. Med.* 81: 85–94.
15. Strassmann, G., V. Patil-Koota, F. Finkelman, M. Fong, and T. Kambayashi. 1994. Evidence for the involvement of interleukin 10 in the differential deactivation of murine peritoneal macrophages by prostaglandin E_2. *J. Exp. Med.* 180: 2365–2370.
16. Portanova, J. P., Y. Zhang, G. D. Anderson, S. D. Hauser, J. L. Masferrer, K. Seibert, S. A. Gregory, and P. C. Isakson. 1996. Selective neutralization of prostaglandin E_2 blocks inflammation, hyperalgesia, and interleukin 6 production in vivo. *J. Exp. Med.* 184: 883–891.
17. Wise, W. C., J. A. Cook, T. Eller, and P. V. Halushka. 1980. Ibuprofen improves survival from endotoxic shock in the rat. *J. Pharmacol. Exp. Ther.* 215: 160–164.
18. Shoup, M., L. K. He, H. Liu, R. Shankar, and R. Gamelli. 1998. Cyclooxygenase-2 inhibitor NS-398 improves survival and restores leukocyte counts in burn infection. *J. Trauma* 45: 215–220.
19. Jacobs, E. R., M. E. Soulsby, R. C. Bone, F. J. Wilson, Jr., and F. C. Hiller. 1982. Ibuprofen in canine endotoxin shock. *J. Clin. Invest.* 70: 536–541.
20. Tilley, S. L., T. M. Coffman, and B. H. Koller. 2001. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *J. Clin. Invest.* 108: 15–23.
21. Rouzer, C. A., P. J. Kingsley, H. Wang, H. Zhang, J. D. Morrow, S. K. Dey, and L. J. Marnett. 2004. Cyclooxygenase-1-dependent prostaglandin synthesis modulates tumor necrosis factor-α secretion in lipopolysaccharide-challenged murine resident peritoneal macrophages. *J. Biol. Chem.* 279: 34256–34268.
22. Taylor, S. S., J. Yang, J. Wu, N. M. Haste, E. Radzio-Andzelm, and G. Anand. 2004. PKA: a portrait of protein kinase dynamics. *Biochim. Biophys. Acta* 1697: 259–269.
23. Cass, L. A., S. A. Summers, G. V. Prendergast, J. M. Backer, M. J. Birnbaum, and J. L. Meinkoth. 1999. Protein kinase A-dependent and -independent signaling pathways contribute to cyclic AMP-stimulated proliferation. *Mol. Cell Biol.* 19: 5882–5891.
24. Fiebich, B. L., S. Schleicher, O. Spleiss, M. Czygan, and M. Hull. 2001. Mechanisms of prostaglandin E_2-induced interleukin-6 release in astrocytes: possible involvement of EP4-like receptors, p38 mitogen-activated protein kinase and protein kinase C. *J. Neurochem.* 79: 950–958.
25. Busca, R., P. Abbe, F. Mantoux, E. Aberdam, C. Peyssonnaux, A. Eychene, J. P. Ortonne, and R. Ballotti. 2000. Ras mediates the cAMP-dependent activation of extracellular signal-regulated kinases (ERKs) in melanocytes. *EMBO J.* 19: 2900–2910.
26. Nochi, H., N. Aoki, K. Oikawa, M. Yanai, Y. Takiyama, Y. Atsuta, H. Kobayashi, K. Sato, M. Tateno, T. Matsuno, et al. 2003. Modulation of hepatic granulomatous responses by transgene expression of DAP12 or TREM-1-Ig molecules. *Am. J. Pathol.* 162: 1191–1201.
27. Gray, T., P. Nettesheim, C. Loftin, J. S. Koo, J. Bonner, S. Peddada, and R. Langenbach. 2004. Interleukin-1β-induced mucin production in human airway epithelium is mediated by cyclooxygenase-2, prostaglandin E_2 receptors, and cyclic AMP-protein kinase A signaling. *Mol. Pharmacol.* 66: 337–346.
28. Knapp, S., S. Gibot, A. de Vos, H. H. Versteeg, M. Colonna, and T. van der Poll. 2004. Expression patterns of surface and soluble triggering receptor expressed on myeloid cells-1 in human endotoxemia. *J. Immunol.* 173: 7131–7134.
29. Fujino, H., S. Salvi, and J. W. Regan. 2005. Differential regulation of phosphorylation of the cAMP response element-binding protein after activation of EP2 and EP4 prostanoid receptors by prostaglandin E_2. *Mol. Pharmacol.* 68: 251–259.
30. Caristi, S., G. Piraino, M. Cucinotta, A. Valenti, S. Loddo, and D. Teti. 2005. Prostaglandin E_2 induces interleukin-8 gene transcription by activating C/EBP homologous protein in human T lymphocytes. *J. Biol. Chem.* 280: 14433–14442.
31. Takeda, K., and S. Akira. 2005. Toll-like receptors in innate immunity. *Int. Immunol.* 17: 1–14.
32. Arend, W. P. 1991. Interleukin 1 receptor antagonist: a new member of the interleukin 1 family. *J. Clin. Invest.* 88: 1445–1451.
33. Abramson, S. B., and A. Amin. 2002. Blocking the effects of IL-1 in rheumatoid arthritis protects bone and cartilage. *Rheumatology* 41: 972–980.
34. Takayama, K., G. Garcia-Cardena, G. K. Sukhova, J. Comander, M. A. Gimbrone, Jr, and P. Libby. 2002. Prostaglandin E_2 suppresses chemokine production in human macrophages through the EP4 receptor. *J. Biol. Chem.* 277: 44147–44154.
35. Treffkorn, L., R. Scheibe, T. Maruyama, and P. Dieter. 2004. PGE_2 exerts its effect on the LPS-induced release of TNF-α, ET-1, IL-1α, IL-6 and IL-10 via the EP2 and EP4 receptor in rat liver macrophages. *Prostaglandins Other Lipid Mediat.* 74: 113–123.
36. Dong, Y. L., D. N. Herndon, T. Z. Yan, and J. P. Waymack. 1993. Blockade of prostaglandin products augments macrophage and neutrophil tumor necrosis factor synthesis in burn injury. *J. Surg. Res.* 54: 480–485.
37. Ertel, W., M. H. Morrison, P. Wang, Z. F. Ba, A. Ayala, and I. H. Chaudry. 1991. The complex pattern of cytokines in sepsis: association between prostaglandins, cachectin, and interleukins. *Ann. Surg.* 214: 141–148.
38. Sacco, S., D. Agnello, M. Sottocorno, G. Lozza, A. Monopoli, P. Villa, and P. Ghezzi. 1998. Nonsteroidal anti-inflammatory drugs increase tumor necrosis factor production in the periphery but not in the central nervous system in mice and rats. *J. Neurochem.* 71: 2063–2070.
39. Sironi, M., M. Gadina, M. Kankova, F. Riganti, A. Mantovani, M. Zandalasini, and P. Ghezzi. 1992. Differential sensitivity of in vivo TNF and IL-6 production to modulation by anti-inflammatory drugs in mice. *Int. J. Immunopharmacol.* 14: 1045–1050.
40. Sakamoto, A., J. Matsumura, S. Mii, Y. Gotoh, and R. Ogawa. 2004. A prostaglandin E_2 receptor subtype EP4 agonist attenuates cardiovascular depression in endotoxin shock by inhibiting inflammatory cytokines and nitric oxide production. *Shock* 22: 76–81.
41. Ejima, K., M. D. Layne, I. M. Carvajal, P. A. Kritek, R. M. Baron, Y. H. Chen, J. Vom Saal, B. D. Levy, S. F. Yet, and M. A. Perrella. 2003. Cyclooxygenase-2-deficient mice are resistant to endotoxin-induced inflammation and death. *FASEB J.* 17: 1325–1327.
42. Murakami, Y., T. Akahoshi, I. Hayashi, H. Endo, S. Kawai, M. Inoue, H. Kondo, and H. Kitasato. 2006. Induction of triggering receptor expressed on myeloid cells 1 in murine resident peritoneal macrophages by monosodium urate monohydrate crystals. *Arthritis Rheum.* 54: 455–462.
43. Sharif, M., J. G. Worrall, B. Singh, R. S. Gupta, P. M. Lydyard, C. Lambert, J. McCulloch, and G. A. Rook. 1992. The development of monoclonal antibodies to the human mitochondrial 60-kd heat-shock protein, and their use in studying the expression of the protein in rheumatoid arthritis. *Arthritis Rheum.* 35: 1427–1433.

検査 1 免疫血清学的検査

- 免疫血清学的検査は，診断，病態の把握，治療効果判定，予後推定に有用となる．

1 血清蛋白分画，膠質反応

血清蛋白は，電気泳動検査で，アルブミン，α_1，α_2，β，γの5つの分画に分かれる．A/G比の低下，γグロブリンの増加は，リウマチ性疾患・膠原病を疑わせる．関節リウマチ（RA），Sjögren症候群（SjS），全身性エリテマトーデス（SLE）などのほか，慢性炎症性疾患，肝疾患でも多クローン性にγグロブリンは増加する．

α_2からγ分画に異常なピーク（M蛋白）を認める場合には，免疫電気泳動検査を行う．

膠質反応検査であるチモール混濁試験（TTT）および硫酸亜鉛混濁試験（ZTT）の高値は，それぞれIgM高値，IgG高値を示す．

2 免疫グロブリン，クリオグロブリン

免疫グロブリンは，IgG，IgA，IgM，IgDおよびIgEの5つのサブクラスに分けられる．リウマチ性疾患・膠原病では多クローン性のIgG増加を示す．一方，原発性および二次性免疫不全症，ネフローゼ症候群，蛋白漏出性胃腸症，ステロイド薬服用時には低下する．

低温で沈降し，37℃で再溶解する免疫グロブリンがクリオグロブリンであり，寒冷時にさまざまな症状を起こす．クリオグロブリン血症は，本態性と膠原病など基礎疾患を持つ二次性に大別される．

3 補　体

C3とC4を定量するか，CH50を測定する．

a．補体低下

SLE活動期，悪性関節リウマチ（MRA），クリオグロブリン血症，肝硬変．

b．補体上昇

RA，リウマチ熱，結節性多発性動脈炎（PN），Behçet病，サルコイドーシス．

4 免疫複合体

抗C1q抗体法，C1q法，抗C3d抗体法などの測定法がある．免疫複合体測定はII型アレルギー（免疫複合体病）の診断，疾患活動性の判定に有用である．RA，SLE，SjS，血管炎症候群，クリオグロブリン血症，Behçet病，サルコイドーシスなどで，関節炎，血管炎，腎炎，発熱，皮膚症状（紅斑，皮膚潰瘍），血液障害（白血球減少，血小板減少）などと関連．

5 リウマトイド因子（RF）

ヒトIgGまたはウサギIgGに対する自己抗体で，主にIgM型のRFを定量法で測定．RAの80％前後で陽性となり，ACRのRA診断基準にも取り上げられ，診断的意義は高い．一方，RA以外の膠原病，慢性肝疾患，感染症，高齢者，健常人などでもときに陽性となる．RF価はRAの活動性と並行することが多く．MRAなどの関節外症状を有するRAで高値となる．

そのほかに下記のRF検出法がある．

① CARF：ガラクトース欠損IgGを抗原とし，全てのクラスのRFを検出．

② IgG-RF：RAでの陽性率は高くないが，MRAで陽性となる．

6 抗核抗体（ANA）

間接蛍光抗体法によるスクリーニングを行い，抗体価，染色型を参考にして，特異自己抗体を検索する（図 1）.

a. 抗体価

ANA 抗体価は，40 倍以上を陽性とすることが多い．この基準では健常人の 10～20％が陽性となるが，多くの例では特異自己抗体は検出されない．混合性結合組織病（MCTD）において，全例が ANA 高力価陽性を示す．SLE，強皮症（SSc），多発性筋炎・皮膚筋炎（PM/DM），SjS においても ANA の陽性率，抗体価ともに高い例が多い．抗 SS-A 抗体，抗 Jo-1 抗体では ANA 陰性の場合もある．ANA 抗体価は，経過中の変動は少ない．また，健常人，高齢者，高 γ グロブリン血症を伴う患者で陽性となることがある．

b. 染色型

ANA の染色型には，均質型，辺縁型，斑紋型，核小体型，離散斑紋型，PCNA 型などがあり，染色型から対応抗原が推定できる．また，ANA 陰性で細胞質染色を示す細胞質型のパターンも検出される．対応抗原から特異自己抗体が推定される．

c. ANA の臨床的意義

疾患に特異性の高い疾患標識自己抗体と臨床像と関連する症状特異自己抗体がある（表 1）．しかし，自己抗体が陰性でも疾患を否定できない．

1）LE 因子

LE 因子は，DNA-ヒストン複合体の不溶性成分に対する自己抗体である．白血球核に LE 因子と補体が結合して，ヘマトキシリン体となり，好中球が貪食したものが LE 細胞現象である．SLE で高頻度かつ高力価に認められ，活動期に高値．プロカインアミドやヒドララジンなどによる薬剤誘発性ループスでも高頻度に検出．

2）抗 DNA 抗体

SLE に特異性が高く診断上有用．抗 dsDNA 抗体価は，疾患活動性とよく相関.

3）抗 Sm 抗体

SLE に特異性が高い．本抗体陽性例では，ほぼ全例が抗 U1 RNP 抗体も陽性.

4）抗 Scl-70（DNA トポイソメラーゼ I）抗体

SSc に特異性が高く，皮膚硬化が全身におよび食道病変，肺線維症などの内臓病変も高度なびまん型皮膚硬化を特徴とする.

5）抗セントロメア抗体

CREST 症候群に特異性が高く，限局型皮膚硬化を伴う SSc と関連する．原発性胆汁性肝硬変，SjS，SLE においても陽性検出.

6）抗 Jo-1 抗体

PM/DM に検出され，陽性例では筋炎に加え，間質性肺炎，多発関節炎を合併.

7）抗 U1 RNP 抗体

SLE，SSc，PM/DM に広く検出されるが，特に膠原病の重複症状を示す例で高率．MCTD では高力価陽性となり，診断上必須項目である.

8）抗 SS-B 抗体

SjS に疾患特異性が高い．眼・口腔乾燥症状を合併する SLE にも見出される．陽性例ではほぼ全例が抗 SS-A/Ro 抗体も陽性.

9）抗 SS-A 抗体

SjS，SLE でしばしばみられ，最も高頻度である．乾燥症状と関連.

7 ANCA の臨床的意義

ANCA は好中球を抗原とした間接蛍光抗体法で，細胞質がびまん性顆粒状染色を示す c-ANCA（PR-3ANCA）と核の周辺が染色される p-ANCA（MPO-ANCA）に分類（図 1）.

PR3-ANCA は，Wegener 肉芽腫症（WG）に特異的，未治療や活動期 WG では高率に陽性．抗体価は疾患活動性を反映.

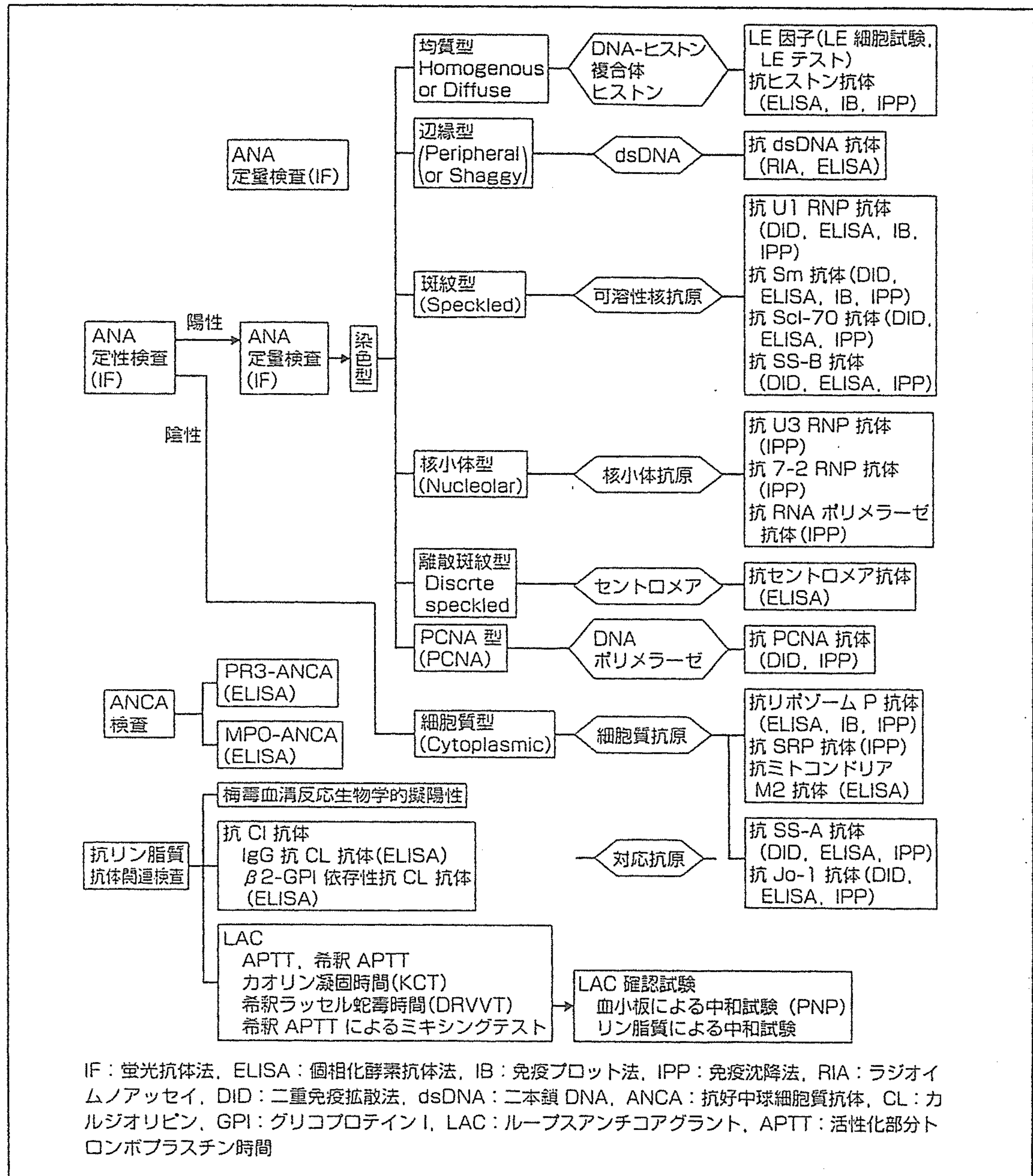

図1 主な自己抗体の検査法

MPO-ANCA は，特発性半月体形成性糸球体腎炎，アレルギー性肉芽腫性血管炎(Churg-Strauss 症候群)，顕微鏡的多発動脈炎で陽性．肺腎症候群(間質性肺炎や肺胞出血，壊死性半月体形成性腎炎)で陽性率が高い．MPO-ANCA は，血管炎を伴う SLE や SSC，RA などでも陽性となる．

8 抗リン脂質抗体の臨床的意義

抗リン脂質抗体は，抗カルジオリピン抗体あるいはカルジオリピン以外のリン脂質，またはリン脂質結合蛋白を認識する自己抗

表 1　膠原病患者血清中に見出される主な疾患標識自己抗体（抗核抗体）

疾　患	陽性頻度
全身性エリテマトーデス（SLE）	
LE 因子	50～60%
抗 dsDNA 抗体	40～70%
抗 Sm 抗体	15～30%
抗リボゾーム P 抗体	10%
抗 PCNA 抗体	3～5%
全身性硬化症（SSc）	
抗 Scl-70（DNA トポイソメラーゼ I）抗体	15～30%
抗セントロメア抗体	20～30%
抗 U3RNP 抗体	4～7%
抗 7-2 RNP 抗体	3～4%
抗 RNA ポリメラーゼ I-III 抗体	< 5%
多発性筋炎および皮膚筋炎（PM/DM）	
抗アミノアシル tRNA 合成酵素抗体	
抗 Jo-1 抗体	20～30%
抗 PL-7 抗体	< 5%
抗 PL-12 抗体	< 5%
抗 OJ 抗体	< 5%
抗 EJ 抗体	< 5%
抗 KS 抗体	< 5%
抗 SRP 抗体	< 5%
抗 Mi-2 抗体	5～10%
overlap 症候群/混合性結合組織病（MCTD）	
抗 U1 RNP 抗体	100%（MCTD）
抗 U2 RNP 抗体・抗 U1/U2 RNP 抗体	< 5%
抗 Ku 抗体	50%（SSc-PM OL）
Sjögren 症候群（SjS）	
抗 SS-B 抗体	20～30%
抗 SS-A 抗体	50～70%

体とループスアンチコアグラント（LAC）の総称である（図 1）．リン脂質結合蛋白を対応抗原とする依存性抗カルジオリピン（β_2GP I）抗体が抗リン脂質抗体症候群（APS）と関連．

9 臓器特異的自己抗体

臓器特異的自己免疫疾患では，臓器特異的自己抗体が診断上参考になる．

10 リンパ球サブセット

免疫担当リンパ球は，T 細胞，B 細胞，NK 細胞に分けられ，さらに表面抗原により分類される．膠原病・自己免疫疾患，ウイルス感染症，造血器腫瘍の診断，病態解析，病型分類に，有用である．

11 HLA 検査

HLA は白血球表面上のヒト主要組織適合抗原であり，クラス I（HLA-A, B, C）とクラス II（HLA-DR, DQ, DP）に分類される．強直性脊椎炎（HLA-B27），Reiter 症候群（HLA-B27），Behçet 病（HLA-B51）などでは，HLA 型と相関．

【諏訪　昭】

疾患と治療 7 混合性結合組織病，Overlap 症候群

- 1人の患者に複数の膠原病の特徴的な症状が認められることがあり，これを膠原病の重複現象という．
- Overlap 症候群（OL）では，複数の膠原病の分類（診断）基準を完全に満たす．
- 混合性結合組織病（MCTD）では，複数の膠原病の分類（診断）基準を完全に満たさず，血清中に抗 U1 RNP 抗体が高抗体価で検出される．

MCTD

1 症　状

①レイノー現象：ほぼ全例に認められ，初診時の数ヵ月～数年前から出現し，初発症状となる．

②指の腫脹（sausage like finger），手背の腫脹（swollen hands）：MCTD に特徴的で，高率に認められる．手指に限局した皮膚硬化や指の腫脹は強皮症（SSc）の初期（浮腫期）でもみられるが，MCTD では全経過を通じて認められる．

③多関節炎：関節リウマチ（RA）の関節炎より軽度であり，骨破壊が見出されることはまれである．SSc の様相が強い例では，手指関節の変形と骨破壊を伴う場合がある．

④肺線維症：慢性肺線維症が最も多い．通常は軽症である．

⑤肺高血圧症（PH）：PH の病態は不可逆的で，進行性である．MCTD では PH の合併率が高い．

⑥腎症：一般に軽症のものが多い．

⑦神経症状：中枢神経障害（無菌性髄膜炎，痙攣，横断性脊髄炎，小脳失調），末梢神経障害（三叉神経障害，手根管症候群）．無菌性髄膜炎の頻度が高い．

⑧その他：SLE や PM/DM のコンポーネントは経過中に消失することが多いのに対し，SSc のコンポーネントは徐々に増える．

2 診断のポイント

1996 年度厚生省 MCTD 班による診断の手引きを参考に診断する（表 1）．PH の診断は，診断の手引きに従う（表 2）．

3 治療のしかた

症例により異なった症状を合わせもつため，病型に応じて治療を選択する．

MCTD の死因は，PH，呼吸不全，心不全が上位を占め，これらの治療が重要である．

OL

1 症　状

SLE，SSc，PM/DM の各所見が同一患者に同時にあるいは経過とともにみられる．

▼トピックス▼

難治病態である PH に対して，種々の血管拡張療法が開発されつつある．プロスタサイクリン（エポプレステノール）持続静注療法は，最も強力な作用を有し，高い有効性を示す．エンドセリン受容体拮抗薬（トラクリア）は，経口投与が可能で，軽・中等症 PH を含めた広い適応が期待されている．

表 1　MCTD 診断の手引き（1996 年改訂）

MCTD の概念：SLE, SSc, PM/DM などにみられる症状や所見が混在し，血清中に抗 U1 RNP 抗体がみられる疾患である．

Ⅰ. 共通所見	1. レイノー現象
	2. 指ないし手背の腫脹
Ⅱ. 免疫学的所見	抗 U1 RNP 抗体
Ⅲ. 混合所見	
A. SLE 様所見	1. 多関節炎
	2. リンパ節腫脹
	3. 顔面紅斑
	4. 心膜炎または胸膜炎
	5. 白血球減少 (4,000/μl 以下) または血小板減少 (10 万/μl 以下)
B. SSc 様所見	1. 手指に限局した皮膚硬化
	2. 肺線維症，拘束性換気障害 (%VC = 80% 以下) または肺拡散能力低下 (% DLco = 70% 以下)
	3. 食道蠕動低下または拡張
C. PM 様所見	1. 筋力低下
	2. 筋原性酵素 (CK) 上昇
	3. 筋電図における筋原性異常所見

診断：
1. Ⅰの 1 所見以上が陽性.
2. Ⅱの所見が陽性
3. ⅢのA, B, C 項のうち，2 項以上につき，それぞれ 1 所見以上が陽性．以上の 3 項を満たす場合を MCTD と診断する．

（厚生労働省 MCTD 班）

表 2　MCTD における肺高血圧症 (PH) 診断の手びき

Ⅰ. 臨床および検査所見
1）労作時の息切れ
2）胸骨左縁収縮性拍動
3）第Ⅱ肺動脈音の亢進
4）胸部 X 線像で肺動脈本幹部の拡大あるいは左第 2 弓突出
5）心電図上右室拡大あるいは右室負荷
6）心エコー上右室拡大あるいは右室負荷

Ⅱ. 肺動脈圧測定
1）右心カテーテルで肺動脈平均圧が 25 mmHg 以上
2）超音波ドプラ法による右心系の圧が右心カテーテルの肺動脈平均圧 25 mmHg 以上に相当

診断：MCTD の診断基準を満たし，Ⅰの 4 項目以上が陽性，あるいはⅡのいずれかの項目が陽性の場合，PH ありとする．Ⅰの 3 項目陽性の場合，PH 疑いとする．

除外項目：　1）先天性心疾患，2）後天性心疾患，3）換気障害性肺性心

（西間木友衛：リウマチ 31：159-166, 1991）

各疾患の組み合わせはさまざまであるが，SLE と SSc の組み合わせが最も多い．女性に多く 40 歳代に高頻度（MCTD とほぼ同じ年齢，性別分布）である．

2 診断のポイント

SLE, SSc, PM の分類（診断）基準に従う．

3 治療のしかた

病型に応じて治療を選択する．MCTD に比して OL の予後は不良である．

【諏訪　昭】

疾患と治療 9

二次性アミロイドーシス

- AA蛋白の沈着により臓器障害をきたす予後不良な病態であり，関節リウマチ(RA)の合併症として重要である．
- 十二指腸粘膜生検でのAA蛋白検出の診断的意義が高い．
- RAの治療に加え，免疫抑制薬・調節剤，ステロイド薬投与や，対症療法として透析療法，中心静脈栄養，抗生物質投与を行う．

1 症状

a．疾患概念

AA蛋白が身体諸臓器の間質に沈着することにより機能障害を起こす．二次性アミロイドーシスの基礎疾患として，RAは重要である．アミロイドーシス発症例では，RA進行例が多い．

b．臨床症状

初期症状は，消化器症状(下痢，腹痛，悪心，嘔吐，腹部膨満感)，腎症状(腎機能障害，蛋白尿，血尿)，心症状(心不全，高血圧，不整脈)，その他の症状(体重減少，発熱，貧血，甲状腺機能低下)である．経過中，心不全，腎障害は進行し，腎不全に至る．低蛋白血症，から敗血症や肺炎を併発する．主な死因は，腎不全，心不全と感染症である．

2 診断のポイント

消化管や腎生検によりAA蛋白を証明する．光学顕微鏡では，AA蛋白はコンゴレッド染色により，燈赤色に染まる．偏光顕微鏡では，AA蛋白は緑色複屈折性を示し，過マンガン酸処理によってその染色性と複屈折性が消失する．

1）上部消化管内視鏡検査

十二指腸第2部でのAA蛋白の陽性率が高く，同粘膜生検が最も優れたスクリーニング法である．無症候でもアミロイド沈着が証明される例もある．

2）尿検査

蛋白尿，血尿．

3）心電図

肢誘導低電位差，$V_{1\text{-}3}$誘導のQSパターン，左軸偏位，心房細動，刺激伝導障害．

3 治療のしかた

a．RAの治療

NSAIDs，DMARDSによるRAの炎症のコントロールが重要である．

b．二次性アミロイドーシスに対する治療

1）免疫抑制薬・調節剤

リウマトレックス，ブレジニン，イムラン，エンドキサンなどを投与する．

[処方例]

○リウマトレックス　4～8mg/週

2）ステロイド薬

プレドニン5～10mg/日やステロイドパルス療法を行う．

[処方例]

○ソル・メドロール1g/日，静注，連続3日間

○その後プレドニゾロン60mg/日（約1mg/体重kg）(分3)，毎食後，

c．対症療法

腎不全に対して血液透析を行う．絶食と中心静脈栄養により腸管の安静を図り，水分，カロリー補給，電解質補正を行う．低蛋白血症に対して蛋白製剤を投与する．下痢症状に止痢剤はしばしば無効で，サラゾピリンが有効なこともある．貧血に対して輸血を行う．感染症に対して抗生物質を投与する．

【諏訪　昭】

疾患と治療 10 成人 Still 病

- 成人 Still 病(ASD)は，若年性関節リウマチの急性発症型(Still 型)が成人に発症したもの．
- 発熱，関節痛，定型的皮疹を特徴とする．
- 不明熱の鑑別として重要．

1 症 状

1) 発熱

弛張熱が突然出現し，数時間で消失する．

2) 関節痛，関節炎

近位指節間(PIP)関節，中手指節間(MP)関節，手，膝，股，肩関節にみられる．一過性のこともあるが，慢性関節炎もある．

3) 定型的皮疹

有熱時に体幹や四肢近位部にサーモンピンクの皮疹が出現し，解熱時には消失する．皮膚を線状に強くこすると，その線上に断続的な隆起疹がみられる(ケブネル現象)．

2 診断のポイント

膠原病，悪性腫瘍，感染症を除外する(表 1)．

3 治療のしかた

a. NSAIDs

NSAIDs を用いる．NSAIDs による肝不全や血管内凝固症候群に注意する．

［処方例］

○ロキソニン 180 mg/日(分 3，毎食後)

b. ステロイド薬

半数以上の例ではステロイド薬を要する．少数例では，プレドニゾロン少量でも有効であるが，無効例では大量投与を行う．

c. DMARDs

［処方例］

○プレドニン 150 mg/日(分 3，毎食後)

○リウマトレックス 4～8 mg/週

表 1　成人 Still 病の診断基準

【診断項目】	
1) 大基準	a) 39℃以上の発熱が 1 週間以上持続する
	b) 2 週間以上持続する関節痛
	c) 定型的皮疹
	d) 白血球増多：10,000/μl 以上，顆粒球 80％以上
2) 小基準	a) 咽頭痛
	b) リンパ節腫脹
	c) 肝機能障害
	d) RF や抗核抗体が陰性
3) 除外疾患	a) 感染症(特に敗血症，伝染性単核症)
	b) 悪性腫瘍(特に悪性リンパ腫)
	c) リウマチ性疾患(特に結節性多発動脈炎，関節外症状を伴うリウマチ性血管炎)
4) 参考項目	フェリチンが高値(正常の 5 倍以上)を示す

【判定】大基準 2 つ以上を含む 5 項目以上の大・小基準を満たし，除外項目を否定できる場合成人 Still 病と診断する．

(厚労省自己免疫疾患の病因・病態解析と新たな治療法の開発に関する調査研究班)

【諏訪　昭】

疾患と治療 15 リウマチ性多発筋痛症

- リウマチ性多発筋痛症(PMR)は，躯幹近位筋群の激しい痛みとこわばり，炎症反応亢進を主症状とする原因不明の炎症性疾患．
- 高齢者に好発する．
- 側頭動脈炎(TA)を合併しやすい．

1 症　状

発症年齢は60歳以上で，平均70歳である．女性に多い(男女比は1:2)．筋症状は，躯幹近位筋(項頸部，肩甲帯，上腕，腰背部，大腿)にみられる．筋痛(自発痛，運動痛，把握痛)とこわばりが主で，原則として筋力低下，筋萎縮はない．症状は急激に発症し，通常は対称性である．肩関節痛がしばしばみられ，まれに関節炎を伴う．発熱，体重減少，全身倦怠感を認める．

2 診断のポイント

a. 検　査

赤沈亢進(> 40 mm/時)，CRP上昇(> 10 mg /dl)を特徴とする．白血球増加，貧血，血小板増加もみられるが，筋原性酵素は正常である．リウマトイド因子や抗核抗体は陰性で，X線所見も正常である．

b. 診　断

診断は，診断基準(表1)によるが，血清反応陰性RA，PM/DM，SLE，PN，線維筋痛症候群などの膠原病，悪性腫瘍，感染症などを鑑別する．PMRの20～30%にTAを合併し，TAの50～70%にPMRを合併する．

表1　PMRの診断基準

1. 両側性肩の疼痛および(または)こわばり
2. 発症2週間以内
3. 赤沈40 mm/時以上
4. 朝のこわばり1時間以上
5. 年齢65歳以上
6. うつ状態および(または)体重減少
7. 両側性上腕部圧痛

上記診断基準項目7項目中3項目を満足する場合，または少なくとも1項目と側頭動脈炎を示す臨床的あるいは病理組織学的異常が共存する場合にはprobable PMRとしてよい．

3 治療のしかた

ステロイド薬が奏功する．奏功しない場合は診断を疑う．初期量として少量(プレドニゾロン10～20 mg/日)を2～4週間継続．臨床症状・検査所見を参考に，以後2～4週毎に10%ずつ減量する．維持量(5 mg/日)を1～2年間継続する．ステロイド中止可能例と長期治療を要する例や再発例もある．TA合併例ではステロイド大量療法を行う．

[処方例]

○プレドニゾロン20 mg/日(分3)

○タケプロン15 mg/日(分1，朝食後)

▼トピックス▼

【線維筋痛症候群】

筋・骨格系など関節外組織の疼痛とこわばり，疲労感を主症状とする症候群である．基礎疾患のない場合，臨床検査で特徴的な異常はみられず，自覚症状と触診による特異的部位での圧痛点から診断される．抗炎症療法は無効で，抗うつ薬や睡眠薬が，顕著な症状である睡眠障害による疲労に有効である．

【諏訪　昭】

ステロイド性骨粗鬆症の海外の予防・治療ガイドライン

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Summary

ステロイド性骨粗鬆症は患者数が多く，若年者や男性にも発症するため社会的影響が大きい．骨量の減少や骨折の増加はステロイド開始から6ヵ月以内にすでにおきるので予防と治療が大事である．予防と治療に関するガイドラインが米国リウマチ学会で発表されて以来，英国，カナダ，オーストラリア，そして日本でつぎつぎと発表された．各ガイドラインではステロイド投与量や骨密度値など，薬物による治療的介入の目安が若干異なっている．薬物療法の中心は，骨量減少と骨折の一次，二次予防において有効性が立証されているビスフォスフォネートである．

Lecture points

- ステロイド投与開始後6ヵ月以内の骨量減少と骨折リスクの増加がみられるので，一次，二次予防とも重要である．
- 海外の主な管理・治療ガイドラインは，米国，英国，カナダ，オーストラリアから発表されている．
- 対象となる症例のステロイド投与量はプレドニゾロン5〜7.5 mg/日以上，3ヵ月以上の内服が目安となる．
- 薬物療法による予防治療的介入が必要となる危険因子として，既骨折，高齢，低骨密度があげられている．
- 薬物療法の中心はビスフォスフォネートである．

Key words

ステロイド性骨粗鬆症　一次予防　二次予防　ガイドライン　ビスフォスフォネート

はじめに

ステロイド（glucocorticoid：GC）薬は小児から老年者に至るまで，さまざまな疾患の治療に用いられているので，ステロイド性骨粗鬆症（glucocorticoid-induced osteoporosis：GCOP）の患者数は原発性骨粗鬆症についで多い．若年者や働き盛りの年齢層における骨折の増加や日常生活機能障害が社会に与える影響は大きい．骨量減少率は，GC開始後はじめの数ヵ月間は8〜12%と高く，その後は年2〜4%の割合で減少する．したがって一次予防が重要となる．最近ではGCOPの

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- 英国の研究では，プレドニゾロン2.5 mg/日以下の少量投与でも骨折危険率の増加がみられた．また，GC累積投与量と腰椎骨密度は逆相関することが報告されている．
- わが国の検討では，骨折例と非骨折例を効率よく分離できる骨密度のカットオフ値はGC投与症例で76.8%であり，原発性骨粗鬆症（70%）にくらべて骨折閾値が高いことが示されている．

予防・治療ガイドラインが海外やわが国で作成されている[1]．

本稿では，予防・治療ガイドラインの根拠となったGCOPに関するエビデンスをまとめ，海外における代表的ガイドラインとして，米国，英国，カナダ，オーストラリアのガイドラインの要旨，特色や，わが国のガイドラインを含めた各ガイドラインの相違を概説する．

1．骨折，骨量減少に関するエビデンス

1）骨折率

GC服用者の骨折率は非服用者の約2倍高い（相対危険率＝1.91）と報告され，とくに脊椎骨折（相対危険率＝2.60），大腿骨頸部骨折（相対危険率＝2.01）の発生率が高く，前腕骨では有意な増加はみられていない[2]．

2）GC投与量と期間

骨折危険率とGC投与量の関連では，英国のGeneral Practice Research Databaseによると，脊椎骨折はプレドニゾロン（PSL）2.5 mg/日以下の少量服用者でも骨折危険率の増加がみられた（相対危険率PSL＜2.5 mg；1.55，2.5〜7.5 mg；2.59，＞7.5 mg；5.18）[2]．しかし，前腕骨の骨折危険率増加はPSL 7.5 mg以上の投与例でのみ認められた．したがって，性別を問わず脊椎，非脊椎骨折危険率ともに増加するのはPSL 7.5 mg/日以上の投与例であるといえる．

骨密度との関連ではGC累積投与量と腰椎骨密度は逆相関することが報告されており，PSL 10 g以上の投与例では骨密度が90%以下に減少するとの報告が多い[2]．また，GC開始後，腰椎骨密度の経時的変化を検討した成績では，GC開始3〜6ヵ月後ですでに骨密度減少がおき，とくにPSL 20 mg/日以上の服用例では投与後3ヵ月で6%以上の骨密度減少がみられた．また，脊椎骨折の増加もGC投与3〜6ヵ月後で明らかであった．

以上の事実から，GCOPの予防・治療ガイドラインはPSL換算5〜7.5 mg/日以上，3〜6ヵ月以上投与中あるいは投与予定の症例を対象としている場合が多い．

3）骨折閾値

PSLの累積投与量約14 gの症例では平均4.7%の腰椎骨密度減少がみられるが，このときの脊椎骨折の相対危険率は2.86〜3.05である．閉経後骨粗鬆症患者では同程度の骨密度減少のときの骨折危険率は1.48と約1/2であった[2]．また，GC服用気管支喘息患者と原発性骨粗鬆症患者の骨折閾値は1.173 vs 0.979と，GCOPでは高い骨密度で骨折がおきていると報告されている[3]．わが国の検討では，骨折例と非骨折例を効率よく分離できる骨密度のカットオフ値はGC投与症例で76.8%と，原発性骨粗鬆症の70%より高かった[4]．以上の事実は，GCOPでは原発性骨粗鬆症にくらべて骨折閾値が高いことを示している．

4）治療薬剤の有効性

GCOPに対して薬物療法が有効であるかは，一次予防（予防），二次予防（治療）効果が立証されているか，あるいは骨量維持・増加効果だけなのか骨折予防効果も証明されているか，の2点が重要である．表❶に大規模比較試験やメタ分析の結果からみた各薬剤のGCOPに対する有効性をまとめる．ビスフォスフォネート製剤はエチドロネート，アレンドロネート，リセドロネートのい

- ビスフォスフォネート製剤はエチドロネート，アレンドロネート，リセドロネートのいずれも一次，二次予防において骨密度減少抑制，骨折予防に有効である．
- 活性型ビタミン D_3 製剤は一次，二次予防において骨密度減少抑制に有効であるが，骨密度増加作用はなく，維持効果にとどまる．

表❶ 治療薬のステロイド性骨粗鬆症に対する有効性

治療薬		脊椎 BMD	脊椎骨折	予防・治療効果	
				一次	二次
ビスフォスフォネート製剤	アレンドロネート	A	A[a]	◎	◎
	周期的エチドロネート	A	A[a]	◎	◎
	リセドロネート	A	A[a]	◎	◎
活性型ビタミン D_3	アルファカルシドール	A	A	○	○
	カルシトリオール	A[b]	A	○	○
カルシトニン		A[b]	A	○	○
カルシウム		NDT	NAE	NDT	NDT
カルシウム＋ビタミンD		A[b]	NAE	NAE	○
フッ化ナトリウム		A	NAE	NAE	○
ホルモン補充療法（tibolone 含む）		A	NAE	NAE	○
テストステロン		A	NAE	NAE	○
副甲状腺ホルモン		A	NAE	NAE	○
ラロキシフェン		no data	no data	NAE	NAE

NAE：not adequately assessed, NDT：not detected, a：not a primary endpoint, b：data inconsistent

ずれも一次，二次予防において骨密度減少抑制，骨折予防に有効である．第1世代と新世代ビスフォスフォネートの有効性に明らかな差はないと考えられる．

活性型ビタミン D_3 製剤は一次，二次予防において骨密度減少抑制に有効であるが，骨密度増加作用はなく，維持効果にとどまる．また，無治療あるいはカルシウム剤治療にくらべて腰椎骨折率の減少効果が期待できる．

カルシトニンの有効性が確認されているのは点鼻製剤の連日投与であり，わが国の筋注用製剤の有効性は無作為化比較試験では確認されていない．ホルモン補充療法は閉経後女性の二次予防において骨密度改善効果が報告されている．

2．海外の予防・治療ガイドライン

1）米国リウマチ学会（ACR）

a．1996年版[5)]

米国リウマチ学会（American College of Rheumatology：ACR）特別委員会より示されたGCOPの予防・治療に関するはじめての勧告である（図❶）．対象はプレドニゾン7.5 mg/日以上，6ヵ月以上内服中，内服予定の症例として，骨粗鬆症骨折の有無，骨密度異常（T score≦－1）の有無で層別化した．すなわち，プレドニゾン7.5 mg/日以上，6ヵ月以上のGC療法は骨密度を低下させ骨折リスクを上昇させるので，骨密度を測定し可能な限り予防処置をとることを勧めている．とくに，骨折がすでにある症例や骨密度減少がある症例ではリスクが高いので薬物療法をおこなう．カ