松田晃	骨髄異形成症候群	臨床血液	50	147-153	2009
松田晃	MDSの診断	臨床血液	50	1477-1488	2009
松田晃	MDSにおける新たな予後スコアリングシステム	血液・腫瘍科	59	20-25	2009
脇本直樹 松田晃	進行期骨髄異形成症候群に対する治療	血液・腫瘍科	58	410-415	2009
松田晃	巨赤芽球性貧血	綜合臨床	58	1722-1727	2009
山口博樹	遺伝子異常による造血不全	最新医学	64	1603-1609	2009
中熊秀喜	エクリツマブによる発作性夜間血色素尿症の治療	血液フロンテイア	19	80-84	2009
中熊秀喜	発作性夜間血色素尿症（PNH）の検査	検査と技術	37	513-518	2009
中熊秀喜	発作性夜間血色素尿症—最近の話題	成人病と生活習慣病	39	808-812	2009
中熊秀喜 花岡伸佳	発作性夜間血色素尿症	総合臨床	58	1736-1740	2009
中熊秀喜	発作性夜間血色素尿症（PNH）に対する治療指針—抗体療法は有効か？	2010-2011　EBM血液疾患の治療		73-78	2009
中熊秀喜	発作性夜間血色素尿症の新しい治療	血液・腫瘍科	59	271-278	2009
中熊秀喜	発作性夜間血色素尿症（PNH）	MEDICO	40	13-16	2009
通山薫	骨髄異形成症候群の形態診断	日本検査血液学会雑誌	10	281-283	2009
松岡亮仁 通山薫	5q-症候群の責任遺伝子とレナリドマイドの分子作用機序	血液・腫瘍科	59	33-39	2009
通山薫	MDSに対する新規治療	綜合臨床	58	1704-1707	2009
辻岡貴之 通山薫	骨髄増殖性腫瘍、骨髄異形成/骨髄増殖性腫瘍	検査と技術（増刊号）	37	1104-1107	2009
通山薫	骨髄異形成症候群	検査と技術（増刊号）	37	1108-1111	2009
田坂大象 通山薫	急性骨髄性白血病	検査と技術（増刊号）	37	1112-1117	2009
通山薫	2008年WHO分類の改正点について	病理と臨床	27	1034-1038	2009
岡部寛 小澤敬也	輸血後鉄過剰症に対するICL670(deferasirox)投与により造血能の回復が得られた骨髄異形成症候群	臨床血液	50	1626-1629	2009
Kataoka K, Nannya YHangaishi A, Imai Y, Chiba S, Takahashi T, Kurokawa M.	Influence of pretransplantation serum ferritin on nonrelapse mortality after myeloablative and nonmyeloablative allogeneic hematopoietic stem cell transplantation.	Biology of Blood and Marrow Transplantation	15	195-204	2009

Hirokawa M, Sawada K, Fujishima N, Kawano F, Kimura A, Watanabe T, Arai A, Matsui T, Nakao S, Urabe A, Omine M, Ozawa K.	Acquire pure red cell aplasia associated with malognant lymphomas : A nationwide cohort study in Japan for PRCA Collaborative Study Group.	Am J Hematol	84	144-148	2009
Mori M, Nakamoto S, Akifuji Y, Tanaka T, Komatsu N, Hatake K, Ozawa, K.	Familial sideroblastic anemia associated with cardiac atrial septal defect.	Am J Hematol	84	451-452	2009
Inomata M, Tagawa H, Guo YM, Kameoka Y, Takahashi N, Sawada K.	MicroRNA-17-92 down-regulates expression of distinct targets in different B-cell lymphoma subtypes.	Blood	113	396-402	2009
Nagai S, Toshima M, Sato K, Mori M, Nagai T, Muroi K, Ozawa K.	Maintenance and preemptive therapy with ganciclovir for cytomegalovirus colitis with extremely high antigenemia in adult T-cell leukemia.	Int J Hematol	89	249-250	2009
Kameoka Y, Takahashi N, Komatsuda A, Tagawa H, Hamai K, Hirokawa M, Wakui H, Ichinohasama R, Sawada K.	Kidney-limited intravascular large B cell lymphoma : a distinct variant of IVLBCL? .	Int J Hematol	89	533-537	2009
Kobayashi H, Matsuyama T, Ueda M, Suzuki T, Ozaki K, Mori M, Nagai T, Muroi K, Ozawa K.	Lenalidomide is active in Japanese patients with symptomatic anemia in low- or intermediate-1 risk myelodysplastic syndromes with a deletion 5q abnormality.	Intern Med	48	1629-1633	2009

Komatsuda A, Wakui H, Iwamoto K, Togashi M, Masai R, Maki N, Sawada K.	GATA-3 is upregulated in peripheral blood mononuclear cells from patients with minimal change nephrotic syndrome.	Clin Nephol	71	608-616	2009
Satoh K, Yoshida N, Imaizumi K, Yajima M, Wakui H, Sawada K, Komatsuda A.	Reversible methotrexate-associated lymphoproliferative disorder resembling advanced gastric cancer in a patient with rheumatoid arthritis.	Am J Med Sci	338	334-335	2009
Yamanaka Y, Tagawa H, Takahashi N, Watanabe A, Y G Mei, Iwamoto K, Ymashita J, Saitoh H, Kameoka Y, Shimizu N, Ichinohasama R, Sawada K.	Aberrant overexpression of microRNAs activate AKT signaling via down-regulation of tumor suppressors in natural killer-cell lympoma/leukemia.	Blood	114	3265-3275	2009
Yoshida M, Nishikawa Y, Yamamoto Y, Doi Y, Tokairin T, Yoshioka T, Omori Y, Watanabe A, Takahashi N, Yoshioka T, Miura I, Sawada K, Enomoto K .	Mast cell leukemia with rapidly progressing portal hypertension.	Pathol Int.	59(11)	817-22	2009
Sawada K, Hirokawa M, Fujishima N.	Diagnosis and management of acquired pure red cell aplasia.	Hematol Oncol Clin North Am.	A23(2)	249-59	2009
Masai R, Wakui H, Togashi M, Maki N, Ohtani H, Komatsuda A, Sawada K.	Clinicopathological features and prognosis in immunoglobulin light and heavy chain deposition disease.	Clin Nephrol	71(1)	9-20	2009

Espinoza JL, Takami A, Onizuka M, Sao H, Akiyama H, Miyamura K, Okamoto S, Inoue M, Kanda Y, Ohtake S, Fukuda T, Morishima Y, Kodera Y, Nakao S	NKG2D gene polymorphism has a significant impact on transplant outcomes after HLA-fully-matched unrelated bone marrow transplantation for standard risk hematologic malignancies.	Haematologica	94	1427-1434	2009
Sugimori C, Mochizuki K, Qi Z, Sugimori N, Ishiyama K, Kondo Y, Yamazaki H, Takami A, Okumura H, Nakao S	Origin and fate of blood cells deficient in glycosylphosphatidylinositol-anchored protein among patients with bone marrow failure.	Br J Haematol	147	102-112	2009
Espinoza JL, Takamatsu H, Lu X, Qi Z, Nakao S	Anti-moesin antibodies derived from patients with aplastic anemia stimulate monocytic cells to secrete TNF-alpha through an ERK1/2-dependent pathway.	Int Immunol	21	913-923	2009
Takamatsu H, Espinoza JL, Lu X, Qi Z, Okawa K, Nakao S	Anti-moesin antibodies in the serum of patients with aplastic anemia stimulate peripheral blood mononuclear cells to secrete TNF-alpha and IFN-gamma.	J Immunol	182	703-710	2009
Hasegawa D, Manabe A, Yagasaki H, Ohtsuka Y, Inoue M, Kikuchi A, Ohara A, Tsuchida M, Kojima S, Nakahata T.	Treatment of Childhood MDS Study Group Trial (MDS99).	Pediatr. Blood Cancer	53	1011-1015	2009

Fukushima-Shintani M, Suzuki KI, Iwatsuki Y, Abe M, Sugasawa K, Hirayama F, Kawasaki T, Nakahata T.	AKR-501 (YM477) a novel orally-active thrombopoietin receptor agonist	Eur J Haematol	82(4)	247-254	2009
Hidaka T, Shide K, Shimoda H, Kameda T, Toyama K, Katayose K, Kubuki Y, Nagata, K, Takenaka K, Akashi K, Okamura T, Niho Y, Mizoguchi H, Omine M, Ozawa K, Harada M, Shimoda K.	The impact of cytogenetic abnormalities on the prognosis of primary myelofibrosis: a prospective survey of 202 cases in Japan.	Eur J Haematol	83	328-333	2009
Niwa A, Umeda K, Chang H, Saito M, Okita K, Takahashi K, Nakagawa M, Yamanaka S, Nakahata T, Heike T	Orderly Hematopoietic Development of Induced Pluripotent Stem Cells via Flk-1^{+} Hemoangiogenic Progenitors	J Cell Physiol	221	367-377	2009
Niwa A, Umeda K, Awaya T, Yui Y, Matsubara H, Hiramatsu H, Watanabe KI, Adachi S, Itoh T, Uemoto S, Nakahata T.	Successful autologous peripheral blood stem cell transplantation with a double-conditioning regimen for recurrent hepatoblastoma after liver transplantation.	Pediatr Transplant.	13(2)	259-262	2009
Chang H, Yoshimoto M, Umeda K, Iwasa T, Mizuno Y, Fukada SI, Yamamoto H, Motohashi N, Suzuki YM, Takeda S, Heike T, Nakahata T.	Generation of transplantable, functional satellite-like cells from mouse embryonic stem cells.	FASEB J.	23	1907-1919	2009

Higashi A.Y, Ikawa T, Muramatsu M, Economides A.N, Niwa A, Okuda T, Murphy AJ, Rojas J, Heike T, Nakahata T, Kawamoto H, Kita T, Yanagita M.	Direct hematological toxicity and illegitimate chromosomal recombination caused by the systemic activation of $CreER^{T2}$.	J Immunol.	182	5633-5640	2009
Watanabe M, Adachi S, Matsubara H, Imai T, Yui Y, Mizushima Y, Hiraumi Y, Watanabe K, Kamitsuji Y, Toyokuni S, Hosoi H, Sugimoto T, Toguchida J, Nakahata T	Induction of autophagy in malignant rhabdoid tumor cells by the histone deacetylase inhibitor FK228 through AIF translocation.	Int J Cancer	124	55-67	2009
Matsubara H, Watanabe M, Imai T, Yui Y, Mizushima Y, Hiraumi Y, Kamitsuji Y, Watanabe K, Nishijo K, Toguchida J, Nakahata T, Adachi S.	Involvement of extracellular signal-regulated kinase activation in human osteosarcoma cell resistance to the histone deacetylase inhibitor FK228 [(1S,4S,7Z,10S,16E,21R)-7-ethylidene-4,21-bis(propan-2-yl)- 2-oxa-12,13-dithia-5,8,20,23-tetraazabicyclo[8.7.6]tricos-16-ene-3,6,9,19,22-pentone]	J Pharmacol Exp Ther.	328(3)	839-48	2009
Okafuji I, Nishikomori R, Kanazawa N, Kambe N, Fujisawa A, Yamazaki S, Saito M, Yoshioka T, Kawai T, Sakai H, Tanizaki H, Heike T, Miyachi Y, Nakahata T.	Role of the NOD2 genotype in the clinical phenotype of Blau syndrome and early-onset sarcoidosis.	Arthritis Rheum.	60(1)	242-250	2009

Yokoo N, Baba S, Kaichi S, Niwa A, Mima T, Doi H, Yamanaka S, Nakahata T, Heike T.	The effects of cardioactive drugs on cardiomyocytes derived from human induced pluripotent stem cells.	Biochem. Biophys. Res. Com.	387(3)	482-488	2009
Hiraumi Y, Iwai-Kanai E, Baba S, Yui Y, Kamitsuji Y, Mizushima Y, Matsubara H, Watanabe M, Watanabe KI, Toyokuni S, Matsubara H, Nakahata T, Adachi S.	Granulocyte colony-stimulating factor protects cardiac mitochondria in the early phase of cardiac injury.	Am J Physiol Heart Circ Physiol.	296	H823-H832	2009
Kato M, Sanada M, Kato I, Sato Y, Takita J, Takeuchi K, Niwa A, Chen Y, Nakazaki K, Nomoto J, Asakura Y, Muto S, Tamura A, Iio M, Akatsuka Y, Hayashi Y, Mori H, Igarashi T, Kurokawa M, Chiba S, Mori S, Ishikawa Y, Okamoto K, Tobinai K, Nakagama H, Nakahata T, Yoshino T, Kobayashi Y, Ogawa S.	Frequent inactivation of A20 in B-cell lymphomas	Nature	459	712-716	2009

Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, Perizot C, Taflin C, Heike T, Valeyre D, Mathian A, Nakahata T, Yamaguchi T, Nomura T, Ono M, Amoura Z, Gorochov G, Sakaguchi S.	Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor.	Immunity	30	899-911	2009
Nie C, Sato K, Misawa N, Kitayama H, Fujino H, Hiramatsu H, Heike T, Nakahata T, Tanaka Y, Ito M, Koyanagi Y.	Selective infection of CD(4)+ effector memory T lymphocytes leads to preferential depletion of memory T lymphocytes in R5 HIV-infected humanized NOD/SCID/IL-2Rγ^{null} mice.	Virology	394	64-72	2009

Sakai M, Miyazaki Y, Matsuo E, Moriuchi Y, Hata T, Fukushima T, Imaizumi Y, Imanishi D, Taguchi J, Iwanaga M, Tsushima H, Inoue Y, Takasaki Y, Tsuchiya T, Komoda M, Ando K, Horio K, Moriwaki Y, Tominaga S, Itonaga H, Nagai K, Tsukasaki K, Tsutsumi C, Sawayama Y, Yamasaki R, Ogawa D, Kawaguchi Y, Ikeda S, Yoshida S, Onimaru Y, Tawara M, Atogami S, Koida S, Joh T, Yamamura M, Matsuo Y, Soda H, Nonaka H, Jinnai I, Kuriyama K, Tomonaga M.	Long-term efficacy of imatinib in a practical setting is correlated with imatinib trough concentration that is influenced by body size: a report by the Nagasaki CML Study Group.	Int J Hematol	89(3)	319-325	2009
Sakamaki H, Ishizawa K, Taniwaki M, Fujisawa S, Morishima Y, Tobinai K, Okada M, Ando K, Usui N, Miyawaki S, Utsunomiya A, Uoshima N, Nagai T, Naoe T, Motoji T, Jinnai I, Tanimoto M, Miyazaki Y, Ohnishi K, Iida S, Okamoto S, Seriu T, Ohno R.	Phase 1/2 clinical study of dasatinib in Japanese patients with chronic myeloid leukemia or Philadelphia chromosome-positive acute lymphoblastic leukemia.	Int J Hematol	89(3)	332-341	2009

Tojo A, Usuki K, Urabe A, Maeda Y, Kobayashi Y, Jinnai I, Ohyashiki K, Nishimura M, Kawaguchi T, Tanaka H, Miyamura K, Miyazaki Y, Hughes T, Branford S, Okamoto S, Ishikawa J, Okada M, Usui N, Tanii H, Amagasaki T, Natori H, Naoe T.	A Phase I/II study of nilotinib in Japanese patients with imatinib-resistant or -intolerant Ph+ CML or relapsed/refractory Ph+ ALL	Int J Hematol	89(5)	679-688	2009
Doi Y, Sasaki D, Terada C, Mori S, Tsuruda K, Matsuo E, Miyazaki Y, Nagai K, Hasegawa H, Yanagihara K, Yamada Y, Kamihira S.	High-resolution melting analysis for a reliable and two-step scanning of mutations in the tyrosine kinase domain of the chimerical bcr-abl gene.	Int J Hematol	90(1)	37-43	2009
Ishikawa Y, Kiyoi H, Tsujimura A, Miyawaki S, Miyazaki Y, Kuriyama K, Tomonaga M, Naoe T.	Comprehensive analysis of cooperative gene mutations between class I and class II in de novo acute myeloid leukemia.	Eur J Haematol	83(2)	90-98	2009
Furuhata A, Kimura A, Shide K, Shimoda K, Murakami M, Ito H, Gao S, Yoshida K, Tagawa Y, Hagiwara K, Takagi A, Kojima T, Suzuki M, Abe A, Naoe T, Murate T.	p27 deregulation by Skp2 overexpression induced by the JAK2V617 mutation	Biochem Biophys Res Commun	383	411-416	2009
Kamesaki T, Oyamada T, Omine M, Ozawa K, Kajii E.	Cut-off value of red-blood-cell-bound IgG for the diagnosis of Coombs-negative autoimmune hemolytic anemia.	Am J Hematol	84	98-101	2009

Yoshida H, Ishida H, Yoshihara T, Oyamada T, Kuwana M, Imamura T, Morimoto A.	Complications of Evans' syndrome in an infant with hereditary spherocytosis: a case report.	J Hematol Oncol	10	40-44	2009
Hosoki T, Ikuta K, Shimonaka Y, Sasaki Y, Yasuno H, Sato K, Ohtake T, Sasaki K, Torimoto Y, Saito K, Kohgo, Y	Heterogeneous expressions of hepcidin isoforms in hepatoma-derived cells detected using simultaneous LC-MS/MS.	Proteomics -Clinical Applications -	3	1256-1264	2009
Ogata K, Porta MGD, Malcovati L, Picone C, Yokose N, Matsuda A, Yamashita T, Tamura H, Tsukada J, Dan K.	Diagnostic utility of flow cytometry in low-grade myelodysplastic syndromes: a prospective validation study	Haematologica	94	1066-1074	2009
Hanaoka N, Nakakuma H, Horikawa K, Nagakura S, Tsuzuki Y, Shimanuki M, Kojima K, Yonemura Y, Kawaguchi T.	NKG2D-mediated immunity underlying proxysmal nocturnal haemoglobinuria and related bone marrow failure syndromes.	Br J Haematol	146	538-545	2009
Kondo T, Tasaka T, Sano F, Matsuda K, Kubo Y, Matsuhashi Y, Nakanishi H, Sadahira Y, Wada H, Sugihara H, Tohyama K.	Philadelphia chromosome-positive acute myeloid leukemia (Ph+AML) treated with imatinib mesylate (IM): a report with IM plasma concentration and bcr-abl transcripts.	Leuk. Res.	33	e137-e138	2009
Harada H, Watanabe M, Suzuki K, Yanagita S, Suzuki T, Yoshida Y, Kimura A, Tsudo M, Matsuda A, Tohyama K, Taniwaki M, Takeshita K, Takatoku M, Ozawa K.	Lenalidomide is active in Japanese patients with symptomatic anemia in low- or intermediate-1 risk myelodysplastic syndromes (MDS) with a deletion 5q abnormality.	Int. J. Hematol.	90	353-360	2009

Uchibori R, Okada T, Ito T, Urabe M, Mizukami H, Kume A, Ozawa K.	Retroviral vector-producing mesenchymal stem cells for targeted suicide cancer gene therapy.	J Gene Med	11	373-381	2009
Ishiwata A, Mimuro J, Mizukami H, Kashiwakura Y, Takano K, Ohmori T, Madoiwa S, Ozawa K, Sakata Y.	Liver-restricted expression of the canine factor VIII gene facilitates prevention of inhibitor formation in factor VIII-deficient mice.	J Gene Med	11	1020-1029	2009
Masuda S, Ageyama N, Shibata H, Obara Y, Ikeda T, Takeuchi K, Ueda Y, Ozawa K, Hanazono Y.	Cotransplantation with MSCs improves engraftment of HSCs after autologous intra-bone marrow transplantation in nonhuman primates.	Exp Hematol	37	1250-1257	2009
Yoshimoto M, Heike T, Chang H, Kanatsu-Shinohara M, Baba S, Varnau JT, Shinohara T, Yoder MC, Nakahata T.	Bone marrow engraftment but limited expansion of hematopoietic cells from multipotent germline stem cells derived from neonatal mouse testis.	Exp Hematol	37	1400-1410	2009

Sanada M, Suzuki T, Shih LY, Otsu M, Kato M, Yamazaki S, Tamura A, Honda H, Sakata-Yanagimoto M, Kumano K, Oda H, Yamagata T, Takita J, Gotoh N, Nakazaki K, Kawamata N, Onodera M, Nobuyoshi M, Hayashi Y, Harada H, Kurokawa M, Chiba S, Mori H, Ozawa K, Omine M, Hirai H, Nakauchi H, Koeffler HP, Ogawa S.	Gain-of-function of mutated c-CBL tumour suppressor in myeloid neoplasms.	Nature	460	904-908	2009
Oka S, Muroi K, Mori M, Matsuyama T, Fujiwara S, Oh I, Sato K, Kikuchi S, Ueda M, Toshima M, Suzuki T, Ozaki K, Nagai T, Ozawa, K.	Prediction of response to imatinib in patients with chronic myelogenous leukemia by flow cytometric analysis of bone marrow blastic cell phenotypes.	Leuk Lymphoma	50	290-293	2009
Oka S, Nagatsuka Y, Kikuchi J, Yokote T, Hirabayashi Y, Hanafusa T, Ozawa K, Muroi K.	Preferential expression of phosphatidylglucoside along neutrophil differentiation pathway.	Leuk Lymphoma	50	1190-1197	2009

Oka S, Muroi K, Matsuyama T, Sato K, Ueda M, Toshima M, Suzuki T, Ozaki K, Mori M, Takubo T, Nagai T, Hanafusa T, Ozawa K.	Correlation between flow cytometric identification of CD33-positive cells and morphological evaluation of myeloblasts in bone marrow of patients with acute myeloblastic leukemia.	Hematology	14	133-138	2009
Hatano K, Kikuchi J, Takatoku M, Shimizu R, Wada T, Ueda M, Nobuyoshi M, Oh I, Sato K, Suzuki T, Ozaki K, Mori M, Nagai T, Muroi K, Kano Y, Furukawa Y, Ozawa, K.	Bortezomib overcomes cell adhesion-mediated drug resistance through downregulation of VLA-4 expression in multiple myeloma.	Oncogene	28	231-242	2009
Kataoka K, Takahashi T, Iwata H, Seo S, Hangaishi A, Kumano K, Kurokawa M.	Fulminant cytomegalovirus myocarditis after allogeneic bone marrow transplantation: successful cytomegalovirus therapy and mechanical circulatory support for bridge to recovery.	Biol Blood Marrow Transplant	16	129-130	2010
Suzuki N, Yumura-Yagi K, Yoshisa M, Hara J, Nishimura S, Kudoh T, Tawa A, Usami I, Tanizawa A, Hori H, Ito Y, Miyaji R, Oda M, Kato K, Hamamoto K, Osugi Y, Hashii Y, Nakahata T, Horibe K	Outcome of childhood acute lymphoblastic leukemia with induction failure treated by the Japan Association of Childhood Leukemia Study (JACLS) ALL F-protocol.	Pediatr Blood Cancer	54(1)	71-78	2010

Kato I, Umeda K, Awaya T, Yui Y, Niwa A, Fujino H, Matsubara H, Watanabe K, Heike T, Adachi N, Endo H, Mizukami T, Nunoi H, Nakahata T, Adachi S.	Successful treatment of refractory donor lymphocyte infusion-induced immune-mediated pancytopenia by Rituximab	Pediatr Blood Cancer	54	329-331	2010
Sakai H, Ito S, Nishikomori R, Takaoka Y, Kawai T, Saito M, Okafuji I, Yasumi T, Heike T, Nakahata T.	A case of early-onset sarcoidosis with a six-base deletion in the NOD2 gene.	Rheumatology (Oxford)	49	194-196	2010
Morita Y, Kanamaru A, Miyazaki Y, Imanishi D, Yagasaki F, Tanimoto M, Kuriyama K, Kobayashi T, Imoto S, Ohnishi K, Naoe T, Ohno R.	Comparative analysis of remission induction therapy for high-risk MDS and AML progressed from MDS in the MDS200 study of Japan Adult Leukemia Study Group.	Int J Hematol	91	97-103	2010
Ohtake, S, Miyawaki, S, Kiyoi, H, Miyazaki, Y, Okumura, H, Matsuda, S, Nagai, T, Kishimoto, Y, Okada, M, Takahashi, M, Honada H, Takeuchi, J, Kageyama, S, Asou, N, Yagasaki, N, Maeda, Y, Ohnishi, K, Naoe, T, Ohno, R	Randomized Trial of Response-Oriented Individualized versus Fixed Schedule Induction Chemotherapy with Idarubicin and Cytarabine in Adult Acute Myeloid Leukemia: The JALSG AML95 Study	Int J Hematol	91	276-283	2010

Sakamaki H, Miyawaki S, Ohtake S, Ygasaki F, Mitani K, Matsuda S, Kishimoto Y, Miyazaki Y, Asou N, Takahashi M, Ogawa Y, Honda S, Ohno R.	Allogeneic Stem Cell Transplantation versus Chemotherapy as Post-remission Therapy for Intermediate or Poor Risk Adult Acute Myeloid Leukemia: Results of the JALSG AML97 Study	Int J Hematol	91	284-292	2010
Ikuta K, Yersin A, Ikai A, Aisen P, Kohgo Y	Characterization of the interaction between diferric transferrin and transferrin receptor 2 by functional assays and atomic force microscopy.	J Mol Biol	397	375-284	2010
Kataoka K, Seo S, Ota S, Takahashi T, Kurokawa M.	Positron emission tomography in the diagnosis and therapeutic monitoring of posttransplant lymphoproliferative disorder after cord blood transplantation.	Bone Marrow Transplant	45	610-612	2010
Ueno H, Blanck JP, Sidney J , Zurawski SM, Bourdery L, Bentebibel SE, Zurawski G, Nicewander D, Heike T, Nakahata T, Arai K, Arai N, Blankenship D, Sette A, Banchereau1 J	Circulating CD4+ T cells Specific for H5 Hemagglutinin in Healthy Subjects.	J Infectious Diseases			In press
Mizuno Y, Chang H, Umeda K, Niwa A, IwasaT, Awaya T, Fukada S, Yamamoto H, Yamanaka S, Nakahata T, Heike T.	Generation of skeletal muscle stem/progenitor cells from murine induced pluripotent stem cells.	FASEB J.			In press

Matsuda A, Germing U, Jinnai I, Araseki K, Kuendgen A, Strupp C, Iwanaga M, Miyazaki Y, Hata T, Bessho M, Gattermann N, Tomonaga M .	Differences in the distribution of subtypes according to the WHO classification 2008 between Japanese and German patients with Refractory Anemia according to the FAB classification in Myelodysplastic Syndromes	Leuk Res			In press
Nagai T, Ohmine K, Fujiwara S-I, Uesawa M, Sakurai C, Ozawa, K.	Combination of tipifarnib and rapamycin synergistically inhibits the growth of leukemia cells and overcomes resistance to tipifarnib via alteration of cellular signaling pathways.	Leuk Res			In press
Kikuchi J, Wada T, Shimizu R, Izumi T, Akutsu M, Mitsunaga K, Noborio-Hatano K, Nobuyoshi M, Ozawa K, Kano Y, Furukawa Y.	Histone deacetylases are critical targets of bortezomib-induced cytotoxicity in multiple myeloma.	Blood			In press
Sakoe Y, Sakoe K, Kirito K, Ozawa K, Komatsu N.	FOXO3A as a key molecule for all-trans retinoic acid-induced granulocytic differentiation and apoptosis in acute promyelocytic leukemia.	Blood			In press
Ishiwata A, Mimuro J, Mizukami H, Kashiwakura Y, Yasumoto A, Sakata A, Ohmori T, Madoiwa S, Ono F, Shima M, Yoshioka A, Ozawa K, Sakata Y.	Mutant macaque factor IX T262A: A tool for hemophilia B gene therapy studies in macaques.	Thromb Res			In press
Meguro A, Ozaki K, Oh I, Hatanaka, K, Matsu H, Tatara R, Sato K, Leonard WJ, Ozawa, K.	IL-21 is critical for GVHD in a mouse model.	Bone Marrow Transplant			In press

VII.　研究成果の刊行物・別刷

（主なもの）

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Anti-moesin antibodies derived from patients with aplastic anemia stimulate monocytic cells to secrete TNF-α through an ERK1/2-dependent pathway

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Abstract

Antibodies specific to moesin, which are frequently detectable in the serum of patients with aplastic anemia (AA), can induce tumor necrosis factor-α (TNF-α) secretion from monocytes and a human monocytic leukemia cell line THP-1. We investigated the mechanisms responsible for TNF-α secretion from monocytic cells induced by the auto-antibodies that are purified from the sera of AA patients. TNF-α induction by anti-moesin antibodies depended on the amount of cell surface moesin expressed by THP-1 cells. $F(ab')_2$ fragments prepared from the anti-moesin antibodies were able to stimulate THP-1 cells to secrete TNF-α and this stimulatory effect was enhanced by cross-linking of moesins with anti-human IgG $F(ab')_2$ fragment antibodies. Anti-moesin antibodies as well as their $F(ab')_2$ fragments induced the phosphorylation of ERK1/2 in monocytic cells and this effect was suppressed by the addition of an ERK1/2 inhibitor. Moreover, anti-moesin antibody treatment induced the phosphorylation of moesin proteins in the monocytes and THP-1 cells within 30 min. These results indicate that anti-moesin antibodies induce TNF-α secretion from monocytes through the activation of the ERK1/2 pathway provoked by direct binding to moesin on the cells.

Introduction

Acquired aplastic anemia (AA) is a disease characterized by bone marrow (BM) failure and pancytopenia. Although several lines of evidence suggest that T cells play a central role in the pathogenesis of AA (1, 2), the humoral immune response to self-antigens may also be implicated in its pathophysiology. Auto-antibodies specific to hematopoietic cell-derived proteins are frequently detected in the serum of AA patients (3–5). It remains unknown whether such antibodies play a role in the pathophysiology of AA.

Antibodies specific to moesin, a membrane cytoskeleton cross-linking protein, are detectable in the serum of ~40% of patients with AA (6). Several reports have shown that moesin is expressed on the cell surface of peripheral blood T cells and monocytes (7–10). In a recent report, we confirmed these observations and demonstrated that anti-moesin antibodies derived from the serum of AA patients, as well as anti-moesin mAb clone 38/87, can induce such immunocompetent cells to secrete myelosuppressive cytokines *in vitro* (11). Because the PBMC of AA patients were highly sensitive to stimulation with anti-moesin antibodies that induced secretion of tumor necrosis factor-α (TNF-α) and IFN-γ in the previous study, anti-moesin antibodies were thought to contribute to the pathophysiology of AA. Although anti-moesin antibody is a novel type auto-antibody that can stimulate autologous immunocompetent cells to secrete inflammatory cytokines, it is totally unknown how the antibodies activate T cells or monocytes. Intensive analysis using monocytic cell lines which express moesin on the cell surface may help to clarify the molecular mechanisms responsible for anti-moesin antibody-induced cytokine secretion.

To test these hypotheses, this study examined the effect of anti-moesin antibodies purified from AA patients' sera on the signaling pathway which mediates TNF-α secretion from THP-1 cells. The present study shows that anti-moesin antibodies induced the activation of the ERK1/2 pathway in monocytic cells and this effect was mediated by the direct binding of anti-moesin antibodies to moesin on THP-1 cells.

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Materials and methods

Antibodies and reagents

The following antibodies and reagents were used in this study: anti-CD40 mAb (clone 82111), isotype mouse IgG1 and mouse IgG2b (R&D Systems, Minneapolis, MN, USA), FITC-labeled goat anti-mouse IgG (BD PharMingen), mouse anti-phospho ERK1/2 mAb (#9106) and rabbit anti-phospho-ezrin/radixin/moesin polyclonal antibody (pAb) (#3142) were purchased from cell signaling technology; anti-total ERK1/2, anti-active p38 and JNK rabbit pAbs were purchased from Promega. Mouse anti-human mAbs including anti-CD14–FITC, anti-CD40–FITC, anti-CD11c–PE and FITC- or PE-labeled isotype IgG were purchased from BD Biosciences. Mouse anti-human Toll-like receptor 4 (TLR4) antibody was purchased from Abcam Inc. (Cambridge, MA, USA) and mouse anti-human CD43 was from AbD Serotec (Oxford, UK). The secondary antibodies used for western blotting were HRP-labeled goat anti-rabbit IgG (Vector, Burlingame, CA, USA), HRP-labeled anti-mouse IgG (GE healthcare, Little Buckinghamshire, UK) as well as alkaline phosphatase-labeled horse anti-mouse IgG, goat anti-human IgG Fab fragment-specific antibody and goat anti-human IgG $F(ab')_2$ fragment-specific antibodies (Jackson Immuno Research). The JNK inhibitor I (L)-form (JNK I) was purchased from Calbiochem. An ERK1/2-specific inhibitor PD98059, a protease inhibitor cocktail, polymyxin B, BSA, fetal bovine serum (FBS), mouse anti-human α-tubulin antibody (clone B-5-1-2) and FITC-labeled anti-human-IgG Fab fragment antibody were purchased from Sigma. Both anti-moesin mAb clone 38 (Transduction laboratories, Lexington, KY, USA) and anti-moesin mAb clone 38/87 (NeoMarkers, Fremont, CA, USA) labeled with FITC by Immuno-Biological Laboratories Co. Ltd (Gunma, Japan) were used for the detection of moesin by western blotting and by flow cytometry, respectively.

A plasmid encoding moesin small hairpin RNA (shRNA) (pENTR/moesin-shRNA-264) and a corresponding negative control (control pENTR/U6-GW/lacZshRNA) were generous gifts of Gregory M. Kelly from the University of Western Ontario, Ontario, Canada (12).

Purification of anti-moesin antibodies

Serum samples were collected from five AA patients, who showed a high titer of anti-moesin antibodies at the time of diagnosis. Anti-moesin pAbs were isolated as described in a previous report (11) and were used to stimulate THP-1 cells or monocytes. Before using the pAbs in the cell stimulation experiments, the purity of the antibodies was determined by SDS-PAGE and Coomasie Brilliant Blue staining, and their specificity was confirmed by western blotting using human recombinant moesin as a target protein.

Purification of human IgG

Serum samples were obtained from 10 ml of blood from three healthy donors. From each sample, the total IgG fraction was isolated using immobilized protein G column chromatography (Amersham Biosciences). The isolated product was dialyzed, filtered and endotoxin removed using the same way as the purified anti-moesin pAbs were treated (11) and then it was used as isotype control pAbs.

Preparation of Fab fragments and $F(ab')_2$ fragments

Fab fragments and $F(ab')_2$ fragments were prepared from anti-moesin pAbs derived from three different patients with AA and IgG derived from three healthy individuals. Fab fragments were generated as described by Adamczyk *et al.* (13). In brief, 10 μl of papain (at 10 mg ml^{-1} suspension in water) was activated by mixing with 90 μl of freshly prepared activation buffer (1 mM EDTA, 10 mM cysteine, 50 mM sodium phosphate, pH 7.0) and incubated at 37°C for 10 min. The activated papain was added to antibody preparation at a papain/antibodies ratio of 5% (w/w). This mixture was incubated at 37°C for 2 h. Digestion was stopped by the addition of 75 μM iodoacetamide (Sigma Chemical Co.) for 30 min on ice. The digestion product was applied to an immobilized protein G affinity chromatography column and the Fab fragments were separated from Fc portion and undigested IgG by elution with PBS. The Fab fragment preparation was further dialyzed with a Float A-lyzer column (Spectrum Laboratories) in PBS overnight. The purity of generated Fab fragments was confirmed by SDS-PAGE and western blotting using anti-human IgG Fab fragment-specific antibodies. The ability of Fab fragments to bind moesin protein on the surface of immune cells was determined by flow cytometry. The purified Fab fragments were endotoxin free as determined by a limulus amebocyte assay.

$F(ab')_2$ fragments were produced by pepsin cleavage of IgG using an $F(ab')_2$ preparation kit (Pierce Chemical Co.) following the manufacturer's recommendations. The reaction mixture was applied to a protein A column to remove Fc fragments and undigested IgG. The $F(ab')_2$ fragments were further dialyzed with a Float A-lyzer column (Spectrum Laboratories) in PBS overnight and thereafter passed through a 0.20-μm filter. The generation of $F(ab')_2$ fragments was confirmed by SDS-PAGE and immunoblotting. The binding of fragments to moesin on the surface of immune cells was determined by flow cytometry. The purified $F(ab')_2$ fragments were endotoxin free as assessed by a limulus amebocyte assay and were used to stimulate THP-1 cells.

Isolation of monocytes

Monocytes of five healthy donors were isolated by plastic adherence as previously described (11). Briefly, 5×10^6 PBMC per well were distributed into 12-well plates (Corning Inc., Costar Lowell, MA, USA) and allowed to adhere in a 5% CO_2 incubator at 37°C for 2 h in RPMI-1640 supplemented with 10% (v/v) heat-inactivated FBS, 100 U ml^{-1} penicillin–0.1 mg ml^{-1} streptomycin (GIBCO) and 10 μg ml^{-1} polymyxin B (designated thereafter as complete culture medium). Non-adherent cells were removed and the remaining adherent cells on the plates were used as monocytes.

Cell culture and determination of cytokines in culture supernatants

A human monocytic leukemia cell line THP-1 was obtained from the Health Sciences Research Resources Bank (Osaka, Japan). Both monocytes and THP-1 cells were cultured in complete culture medium at 37°C in a humidified 5% CO_2 atmosphere. The cells were incubated in the presence of 5 μg ml^{-1} of anti-moesin pAbs or human IgG derived from healthy donors and