研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yu Ishima, Fumika Yoshida, Ulrich Kragh-Hansen, Kaori Watanabe, Naohisa Katayama, Keisuke Nakajou, Takaaki Akaike, Toshiya Kai, Toru Maruyama and Masaki Otagiri.	Cellular uptake mechanisms and responses to NO transferred from mono- and poly-S-nitrosated human serum albumin.	Free Radic Res.	45	1196-1206	2011
Hijikata M, Matsushita I, Tanaka G, Tsuchiya T, Ito H, Tokunaga K, Ohashi J, Homma S, Kobashi Y, Taguchi Y, Azuma A, Kudoh S, Keicho N	Molecular cloning of two novel mucin-like genes in the disease-susceptibility locus for diffuse panbronchiolitis.	Human Genetics	129	117-128	2011
Sakamoto S, Homma S, Mun M, Fujii T, Kurosaki A, Yoshimura K	Acute exacerbation of idiopathic interstitial pneumonia following lung surgery in 3 of 68 consecutive patients	Intern Med	50	77-85	2011
Aoyagi T, Yamamoto N, Hatta1 M, Tanno D, Miyazato A, Ishii K, Suzuki K, Nakayama T, Taniguchi M, Kunishima H, Hirakata Y, Kaku M, Kawakami K	Activation of pulmonary invariant NKT cells leads to exacerbation of acute lung injury caused by LPS through local production of IFN-γ and TNF-α by Gr-1+ monocytes.	International Immunology	23	97-108	2011
Mariko Baba, Yasuhiro Mehara, Atsuko Matsuya, Tomokazu Nagao, Kazuo Suzuki, Shoji Kawachi.	Levels of Seventeen Differnt Cytokindes in Bronchoalveolar Lavage Fluid Samples from Two Patients with Connective Tissue Diseases and Acute Respiratory Disteress Syndorome.	IRYO	65(8)	440-5	2011
Hideshi Ihara, Tomohiro Sawa, Yusaku Nakabeppu and Takaaki Akaike.	Nucleotides function as endogenous chemical sensors for oxidative stress signaling.	J Clin Biochem Nutr.	48	1-7	2011
S. Kawachi, T. Matsushita, T. Sato, H. Nunoi, H. Noguchi, S. Ota, N. Kanemoto, K. Nakatani, T. Nishiguchi, A. Yuge, H. Imamura, H. Kitajima, K. Narahara, K. Suzuki, T. Miyoshi-Akiyama, T. Kirikae.	Multicenter prospective evaluation of a novel rapid immunochromatographic diagnostic kit specifically detecting influenza A H1N1 2009 virus.	J Clin Virol	51(1)	68-72	2011

研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yuki Kurauchi, Akinori Hisatsune, Yoichiro Isohama, Tomohiro Sawa, Takaaki Akaike, Koichi Shudo and Hiroshi Katsuki.	Midbrain dopaminergic neurons utilize nitric oxide/cyclic GMP signaling to recruit ERK that links retinoic acid receptor stimulation to up-regulation of BDNF.	J Neurochem.	116	323-333	2011
Thuy T.B.Phung, San T. Luong, Shoji Kawachi, Hiroyuki Nunoi, Liem T.Nguyen, Toshinori Nakayama, Kauo Suzuki.	Interleukin 12 and myeloperoxidase(MPO)in Vietnamese children with acute respiratory distress syndrome due to Avian influenza(H5N1)infection	Journal of Infection	62	104-108	2011
Phung TTB, Sugamata R, Uno K, Aratani Y, Ozato K, Kawachi S, Nguyen LT, Nakayama T, Suzuki K.	Key role of RANTES (regulated upon activation normal T-cell expressed and secreted), nonstructural protein1 and myeloperoxidase in cytokine storm induced by influenza virus PR-8(A/H1N1) infection in A549 bronchial epithelial cells.	Microbiol Immunol.	55(12)	874-884	2011
Mina Nakauchi, Tetsushi Yoshikawa, Hidetaka Nakai, Ken Sugata, Akiko Yoshikawa, Yoshizo Asano, Masaru Ihira, Masato Tashiro, Tsutomu Kageyama	Evaluation of reverse transcription loop-mediated isothermal amplification assays for rapid diagnosis of pandemic influenza A/H1N1 2009 virus	Journal of Medical Virology	83(1)	10-15	2011
Mina Nakauchi, Makoto Ujike, Masatsugu Obuchi, Emi Takashita, Ikuyo Takayama, Miho Ejima, Kunihiro Oba, Nami Konomi, Takato Odagiri, Masato Tashiro, Tsutomu Kageyama, the influenza virus surveillance group of Japan.	Rapid discrimination of oseltamivir-resistant 275Y and -susceptible 275H substitutions in the neuraminidase gene of pandemic influenza A/H1N1 2009 virus by duplex one-step RT-PCR assay	Journal of Medical Virology	83(7)	1121-1127	2011
Nakauchi M, Ujike M, Obuchi M, Takashita E, Takayama I, Ejima M, Oba K, Konomi N, Odagiri T, Tashiro M, Kageyama T;	the influenza virus surveillance group of Japan. Rapid discrimination of oseltamivir-resistant 275Y and -susceptible 275H substitutions in the neuraminidase gene of pandemic influenza A/H1N1 2009 virus by duplex one-step RT-PCR assay.	Journal of Medical Virology.	83(7)	1121-27	2011
本間　栄、村松陽子	間質性肺炎を管理・治療する-N-アセチルシステイン(N-acetylcysteine)-	Mebio	28	85-93	2011

研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Phung TTB, Sugamata R, Uno K, Aratani Y, Ozato K, Kawachi S, Nguyen LT, Nakayama T, Suzuki K.	Key role of RANTES (regulated upon activation normal T-cell expressed and secreted), nonstructural protein1 and myeloperoxidase in cytokine storm induced by influenza virus PR-8(A/H1N1) infection in A549 bronchial epithelial cells.	Microbiol Immunol	55(12)	874-884	2011
T. Nagao, K. Suzuki, K. Utsunomiya, M. Matsumura, K. Saiga, P-C. Wang, H. Minamitani, Y. Aratani, T. Nakayama, K. Suzuki.	Direct activation of glomerular endothelial cells by anti-moesin activity of anti-myeloperoxidase antibody.	Nephrology Dialysis Transplantation	26	2752-2760	2011
Hideshi Ihara, Ahmed Khandaker Ahtesham, Tomoaki Ida, Shingo Kasamatsu, Kouhei Kunieda, Tatsuya Okamoto, Tomohiro Sawa and Takaaki Akaike.	Methodological proof of immunochemistry for specific identification of 8-nitroguanosine 3',5'-cyclic monophosphate formed in glia cells.	Nitric Oxide	25	169-175	2011
Takaaki Akaike, Albert van der Vliet and Philip Eaton.	Frontiers in nitric oxide and redox signaling.	Nitric Oxide	25	57-58	2011
Sugino K, Hebisawa A, Uekusa T, Hatanaka K, Abe H, Homma S	Histopathological bronchial reconstruction of human bronchiolitis obliterans.	Pathol Intern	61	192-201	2011
Fujimoto S, Watts RA, Kobayashi S, Suzuki K, Jayne DR, Scott DG, Hashimoto H, Nunoi H.	Comparison of the epidemiology of anti-neutrophil cytoplasmic antibody-associated vasculitis between Japan and the U.K.	Rheumatology (Oxford).	50(10)	1916-20	2011
佐野　剛、卜部尚久、岩田基秀、舘田一博、山口恵三、本間　栄	気管支拡張症に合併した耐性緑膿菌による難治性持続気道感染の1例	Therapeutic Research,	32	22-25	2011
Isobe K, Hata Y, Sakguchi S, Sato K, Sano G, Sugino K, Sakamoto S, Takai Y, Shibuya K, Takagi K, Homma S	The role of fluoro-2-deoxyglucose positron emission tomography for the detection of gastrointestinal tract lesions in patients with lung cancer.	Thoracic Cancer	2	190-195	2011

研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Inoue Y, Aizaki H, Hara H, Matsuda M, Ando T, Shimoji T, Murakami K, Masaki T, Shoji I, Homma S, Matsuura Y, Miyamura T, Wakita T, Suzuki T	Chaperonin TRiC/CCT participates in replication of hepatitis C virus genome via interaction with the viral NS5B protein.	Virology	410	38-47	2011
藤岡俊一郎, 保坂茂, 尾澤直美, 南恵理, 柴田真希, 原久男, 梶尾裕, 岸本美也子, 野田光彦, 河内正治	第25回 重症心疾患を合併した慢性腎不全の2例 国立国際医療研究センター病院 生活習慣病症例検討会から	医療	65(11)	579-584	2011
河内正治	BAL(今月の用語)	医療	65(8)	446	2011
澤智裕, 小野勝彦, 赤池孝章	活性酸素・一酸化窒素によるニトロ化シグナルと抗炎症作用	感染・炎症・免疫	41	12-19	2011
河内正治	「各国の医療事情」.ベトナムの医療事情―厚生労働省科学研究費研究班を通じてのベトナム医療との関わり―.	日本臨床麻酔学会誌	31(4)	620-628	2011
本間　栄、村松陽子、杉野圭史、坂本　晋	IPFの新しい治療戦略.	臨床呼吸生理	43	57-63	2011
河内正治	吸入麻酔薬によるICU Sedation.	臨床麻酔	35(10)	1529-36	2011
今吉理恵子, 岡本竜哉, 永尾潤一, 長環, 松永勝政, 赤池孝章, 上西秀則	植物精油citralのインフルエンザウイルス感染能抑制効果について：Experimental study of a citral on the preventive effect ofinfluenza virus infection.	医学と生物学	155	489-494	2011
佐藤敬太、本間　栄	ANCA関連血管炎の肺病変-MPAの肺病変を中心に	医学のあゆみ	236	765-769	2011
高尾信一、原三千丸、岡崎富男、鈴木和男	ヒト呼吸器系ウイルスの検出における呼吸器系ウイルス多項目同時解析アッセイ（Luminex xTAG Respiratory Viral Panel FAST Assay）の有用性の検討	感染症誌	85	31～36	2011

研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
山越志保、本間　栄、川畑雅照	Multidetector-row CT angiographyが気管支動脈塞栓術に有用であった、びまん性汎細気管支炎に伴う喀血の1例	気管支学	33	256-260	2011
中島典子、佐多徹太郎	鳥インフルエンザウイルスのヒトへの感染とその病態	鶏病研究会会報	47	71-77	2011
杉野圭史、本間　栄	Hermansky-Pudlak症候群と間質性肺炎	呼吸	30	371-378	2011
本間　栄、村松陽子	IPFの治療-2)N-アセチルシステイン(N-acetylcysteine)-.	呼吸器内科	19	575-582	2011
工藤大介、久志本成樹、川上和義	病態生理, 第2章　病理・病態生理「新しい診断と治療のABC 肺炎　改訂第2版」	最新医学別冊		39-45	2011
岡本竜哉, 居原秀, 赤池孝章	喫煙と酸化ストレス：Oxidative stress induced by cigarette smoking.	最新精神医学. 特集号「精神障害者と喫煙」	16	431-494	2011
本間　栄	特発性肺線維症(蜂巣肺)の新しい治療戦略.	順天堂医学	57	68-70	2011
本間　栄	特発性肺線維症の最新治療	難病と在宅ケア	16	32-35	2011
杉野圭史，石田文昭，高井雄二郎，渋谷和俊、植草利公、本間　栄	関節リウマチの治療中に発症した好酸球性肺炎の1例	日胸	70	1167-1175	2011
磯部和順、永田梨耶、太田宏樹、杉野圭史、渋谷和俊，本間　栄	化学療法中に傍腫瘍性辺縁系脳炎を合併した肺小細胞癌の1例	日呼吸会誌	49	602-606	2011
後町杏子、坂本　晋、笹本修一、渋谷和俊、高木啓吾、本間　栄	3D-CTにより診断し、異常血管のみの切除にて治癒した肺底動脈大動脈起始症の1例	日呼吸会誌	49	221-225	2011
本間　栄	Kartgagener症候群における細気管支炎	日本医事新報	4567	50-52	2011

研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
磯部和順、秦　美暢、佐藤敬太、佐野　剛，杉野圭史、坂本　晋、高井雄二郎、本間　栄	末梢血液中の循環細胞を用いたEGFR遺伝子変異検出の検討	肺癌	51	659-693	2011
徳橋芙美子、阪口真之、磯部和順、杉野圭史、羽鳥努、本間　栄	ゲフイチニブ投与前にL858RとT790MのEGFR遺伝子変異を認めた原発性肺癌の1剖検例.	肺癌	51	84-88	2011
村松陽子、杉野圭史、佐藤敬太、坂本　晋、建部順子、盛田俊介、本間　栄	特発性肺線維症におけるNAC単独吸入療法の効果とレドックス制御	臨床呼吸生理	43	51-54	2011
T. Aoyagi, N. Yamamoto, M. Hatta, D. Tanno, A. Miyazato, K. Ishii, K. Suzuki, T. Nakayama, M. Taniguchi, H. Kunishima, Y. Hirakata, M. Kaku, K. Kawakami.	Activation of pulmonary invariant NKT cells lead to exacerbation of acute lung injury caused by lipopolysaccharide through local production of IFN-γ and TNF-α by Gr-1+ monocytes.	International Immunol.	33	442-455	2010
Tomohiro Sawa, Hiorokazu Arimoto, Takaaki Akaike.	Regulation of redox signaling involving chemical conjugation of protein thiols by nitric oxide and electrophiles.	Bioconjug Chem	21	1121-1129	2010
Hidenori YASUDA, Nobuaki YOSHIZAWA, Masaaki MATSUMOTO, Shoji KAWACHI, Kazuo SUZUKI.	Transmission of Pandemic H1N1 Influenza in Japan in 2009: Simulated Measures and Post-Analysis.	EASIAM Conference	6	110-116	2010
Sakamoto S,Homma S,Miyamoto A,Kurosaki A,Fujii T,Yoshimura K	Cyclosporin A treatment in acute exacerbation of idiopathic pulmonary fibrosis.	Intern Med	49	109-115	2010
Hori J, Taniguchi H, Wang M, Oshima M, Azuma M	GITR ligand-mediated local expansion of regulatory T cells contributes to immune privilege of corneal allografts.	Invest Ophthalmol Vis Sci.	51	6556-65	2010
Shigemoto Fujii, Tomohiro Sawa, Hideshi Ihara, Kit I. Tong, Tomoaki Ida, Tatsuya Okamoto, Ahmed Khandaker Ahtesham, Yu Ishima, Hozumi Motohashi, Masayuki Yamamoto, Takaaki Akaike.	The critical role of nitric oxide signaling, via protein S-guanylation and nitrated cyclic GMP, in the antioxidant adaptive response.	J Biol Chem	285	23970-23984	2010

研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tatsuya Okamoto, Shahzada Khan, Kohta Oyama, Shigemoto Fujii, Tomohiro Sawa, Takaaki Akaike.	A new paradigm for antimicrobial host defense mediated by a nitrated cyclic nucleotide.	J Clin Biochem Nutr	46	14-19	2010
Yuka Osaki, Yasuhiro Maehara, Masaki Sato, Akiyoshi Hoshino, Kenji Yamamoto, Tomokazu Nagao, Kazuo Suzuki, Shoji Kawachi.	Analysis of cytokine/chemokine levels in bronchoalveolar lavage fluids from patients with acute respiratory distress syndrome: Increase in IL-6, G-CSF, MCP-1, MIP-1β.	Jap J Intens Care Med	17	179-184	2010
Lijuan Yu, Chie Aoki, Yohko Shimizu, Kazufumi Shimizu, Wei Hou, Fumihiro Yagyu, Xianzi Wen, Masamichi Oshima, Aikichi Iwamoto, Bin Gao, Wenjun Liu, George Fu Gao, Yoshihiro Kitamura	Development of a simple system for screening anti-hepatitis C virus drugs utilizing mutants capable of vigorous replication	Journal of Virological Methods.	69	380-4	2010
Igari H, Segawa S, Watanabe A, Suzuki A, Watanabe M, Sakurai T, Kuroda F, Watanabe M, Tatsumi K, Nakayama M, Nakayama T, Suzuki K, Sato T.	Immunogenicity of a Monovalent Pandemic Influenza AH1N1 Vaccine in Healthcare Workers.	Microbiol Immunol	2010; 54: 618–62454	618–624	2010
Yu Ishima, Shuichi Hiroyama, Ulrich Kragh-Hansen, Toru Maruyama, Tomohiro Sawa, Takaaki Akaike, Toshiya Kai, and Masaki Otagiri.	One-step preparation of S-nitrosated human serum albumin with high biological activities.	Nitric Oxide	23	121-127	2010
Takaaki Akaike, Shigemoto Fujii, Tomohiro Sawa, Hideshi Ihara.	Cell signaling mediated by nitrated cyclic guanine nucleotide.	Nitric Oxide	23	166-174	2010
Kuroda M, Katano H, Nakajima N, Tobiume M, Ainai A, Sekizuka T, Hasegawa H, Tashiro M, Sasaki Y, Arakawa Y, Hata S, Watanabe M, Sata T.	Characterization of quasispecies of pandemic 2009 influenza A virus (A/H1N1/2009) by de novo sequencing using a next-generation DNA sequencer.	PLoS One	23;5(4)	p1-9	2010
Isobe K,Hata Y,Sakamoto S,Takai Y,Homma S	Clinical characterisitics of acute respiratory deterioration in pulmonary fibrosis associated with lung cancer following anti-cancer therapy	Respirology	15	88-92	2010

研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tomizawa K, Nagao T, Kusunoki R, Saiga K, Oshima M, Kobayashi K, Nakayama T, Tanokura M, Suzuki K	Reduction of MPO-ANCA epitopes in SCG/Kj mice by 15-deoxyspergualin treatment restricted by IgG2b associated with crescentic glomerulonephritis.	Rheumatology (Oxford)	49	1245-56	2010
河内正治	新型インフルエンザ(今月の用語)	医療	64(10)	653-654	2010
家研也、吉澤篤人、平野聡、泉信有、放生雅章、杉山温人、小林信行、工藤宏一郎、前原康宏、河内正治、宮越浩一	気管支喘息合併全身麻酔症例の周術期発作に関する検討.	アレルギー	59(7)	831-838	2010
朝山京子、松谷厚子、佐々木立朗、伊藤倫子、前原康宏、河内正治	自発呼吸下に全身麻酔をおこなった外傷性気管損傷の1例	麻酔と蘇生	46(4)	73-75	2010
河内正治、丸谷晶美	人工呼吸中の栄養管理	日本呼吸ケア・リハビリテーション学会誌	20(2)	120-124	2010
藤井重元、澤智裕、赤池孝章.	8-Nitro-cGMPの発見と生理機能の解明	化学と生物	48	22-27	2010
澤智裕、赤池孝章.	毒性説から脱した活性酸素研究-シグナル分子としての活躍-	現代化学	469	34-38	2010
岡本竜哉、赤池孝章.	呼吸器疾患における酸化ストレスと制御シグナルの分子基盤: Molecular mechanisms of nitric oxide- and reactive oxygen species-mediated signalings in the respiratory diseases.	呼吸	29	859-866	2010
岡本竜哉、澤智裕、赤池孝章.	Nitric oxide (NO) およびNO関連物質.	日本臨床増刊号広範囲血液・尿化学検査, 免疫学的検査-4	982	839-842	2010

研究成果の刊行に関する一覧表

書籍

著者氏名	タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
Tatsuya Okamoto, Mohammad Hasan Zaki, Shigemoto Fujii, Tomohiro Sawa, Takaaki Akaike.	Nitric oxide-mediated host immune response and microbial pathogenesis.	Bahar Tunctan	Nitric Oxide Synthase Inhibitors: From Animal Studies to Clinical Implications	American Society for Pharmacology & Experimental Therapeutics (ASPET)	Bethesda, USA	in press	
高病原性鳥インフルエンザの診断・治療に関する国際連携研究班	重症新型インフルエンザ診断と治療の手引-鳥インフルエンザウイルスは、ヒトに感染する-	河内正治	鳥インフルエンザウイルスがヒトに感染するとどうなるか	メディカル・サイエンス・インターナショナル	東京	2013	
河内正治	肺疾患	丸山道夫、山東勤弥、保木昌徳	経腸栄養マニュアル	文光堂	東京	2012	180-186
河内正治	急性呼吸窮迫症候群、急性肺障害	吉澤篤人、杉山温人	レジデントのための呼吸器内科ポケット分区	中山書店	東京	2012	42-49
河内正治	吸入麻酔薬	天羽敬祐	麻酔科学レビュー2011	総合医学社	東京	2012	63-69
河内正治	呼吸障害者の栄養管理は？	東口髙志	重症患者と栄養管理Q&A	総合医学社	東京	2012	157-162
河内正治	呼吸不全用栄養剤	丸山道生、小川哲	PDN Lectures Chapter-2 経腸栄養	PEGドクターズネットワーク	東京	2011	http://peg.or.jp/
河内正治	肺疾患	丸山道夫、山東勤弥、保木昌徳	経腸栄養マニュアル	文光堂	東京	2011	180-186
本間栄	閉塞性細気管支炎	山口　徹、北原光夫、福井次矢編	2011今日の治療指針	医学書院	東京	2011	294-295
本間栄	特発性間質性肺炎	日本呼吸器学会びまん性肺疾患診断・治療ガイドライン作成委員会編	診断と治療の手引き　改訂第2版	南江堂	東京	2011	
坂本晋　本間栄	急性好酸球性肺炎	石原照夫　編	呼吸器疾患ビジュアルブック	学研メジカル秀潤社	東京	2011	181-183
坂本晋　本間栄	慢性好酸球性肺炎	石原照夫　編	呼吸器疾患ビジュアルブック	学研メジカル秀潤社	東京	2011	184-186

研究成果の刊行に関する一覧表

著者氏名	タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
坂本晋　本間栄	アレルギー性気管支肺アスペルギルス症	石原照夫　編	呼吸器疾患ビジュアルブック	学研メジカル秀潤社	東京	2011	187-190
大島正道	SARS	桂義元、河本宏、小安重夫、山本一彦	免疫の辞典	朝倉書店	東京	2011	212
大島正道	種痘	桂義元、河本宏、小安重夫、山本一彦	免疫の辞典	朝倉書店	東京	2011	244
大島正道	垂直感染／水平感染	桂義元、河本宏、小安重夫、山本一彦	免疫の辞典	朝倉書店	東京	2011	255
大島正道	レトロウイルス	桂義元、河本宏、小安重夫、山本一彦	免疫の辞典	朝倉書店	東京	2011	450
大島正道	SARS	桂義元、河本宏、小安重夫、山本一彦	免疫の辞典	朝倉書店	東京	2011	212
大島正道	種痘	桂義元、河本宏、小安重夫、山本一彦	免疫の辞典	朝倉書店	東京	2011	244
大島正道	垂直感染／水平感染	桂義元、河本宏、小安重夫、山本一彦	免疫の辞典	朝倉書店	東京	2011	255
大島正道	レトロウイルス	桂義元、河本宏、小安重夫、山本一彦	免疫の辞典	朝倉書店	東京	2011	450

Inflammation (© 2013)
DOI: 10.1007/s10753-013-9607-1

Low-dose Interferon-α Treatment Improves Survival and Inflammatory Responses in a Mouse Model of Fulminant Acute Respiratory Distress Syndrome

Daisuke Kudo,[1,2] Kazuko Uno,[3] Tetsuji Aoyagi,[4] Yukiko Akahori,[1] Keiko Ishii,[1] Emi Kanno,[5] Ryoko Maruyama,[5] Shigeki Kushimoto,[2] Mitsuo Kaku,[4] and Kazuyoshi Kawakami[1,6]

Abstract—Acute respiratory distress syndrome (ARDS) is accompanied by severe lung inflammation induced by various diseases. Despite the severity of symptoms, therapeutic strategies for this pathologic condition are still poorly developed. Interferon (IFN)-α is well known as an antiviral cytokine and low-dose IFN-α has been reported to show antiinflammatory effects. Therefore, we investigated how this cytokine affected ARDS in a mouse model. C57BL/6 mice received sequential intratracheal administration of α-galactosylceramide (α-GalCer) and lipopolysaccharide (LPS), which resulted in the development of fulminant ARDS. These mice were then treated intranasally with IFN-α and their survival, lung weight, pathological findings, and cytokine production were evaluated. Administration of low-dose IFN-α prolonged survival of fulminant ARDS mice, but higher doses of IFN-α did not. Histological analysis showed that low-dose IFN-α treatment improved findings of diffuse alveolar damage in fulminant ARDS mice, which was associated with reduction in the wet/dry (W/D) lung weight ratio. Furthermore, IFN-γ production in the lungs was significantly reduced in IFN-α-treated mice, compared with control mice, but tumor necrosis factor (TNF)-α production was almost equivalent for both groups. Low-dose IFN-α shows antiinflammatory and therapeutic effects in a mouse model of fulminant ARDS, and reduced production of IFN-γ in the lung may be involved in the beneficial effect of this treatment.

KEY WORDS: ARDS; IFN-α; anti-inflammatory effects; IFN-γ; NKT cells.

[1] Department of Medical Microbiology, Mycology and Immunology, Tohoku University Graduate School of Medicine, 2-1 Seiryo-cho, Aoba-ku, Sendai Miyagi 980-8575, Japan
[2] Department of Emergency Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryo-cho, Aoba-ku, Sendai Miyagi 980-8574, Japan
[3] Louis Pasteur Center for Medical Research, 103-5, Tanakamonzen-machi, Sakyo-ku, Kyoto 606-8225, Japan
[4] Department of Infection Control and Laboratory Diagnostics, Internal Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryo-cho, Aoba-ku, Sendai Miyagi 980-8574, Japan
[5] Department of Science of Nursing Practice, Tohoku University Graduate School of Medicine, 2-1 Seiryo-cho, Aoba-ku, Sendai Miyagi 980-8575, Japan
[6] To whom correspondence should be addressed at Department of Medical Microbiology, Mycology and Immunology, Tohoku University Graduate School of Medicine, 2-1 Seiryo-cho, Aoba-ku, Sendai Miyagi 980-8575, Japan. E-mail: kawakami@med.tohoku.ac.jp

INTRODUCTION

Acute respiratory distress syndrome (ARDS) is characterized as a diffuse lung injury with severe hypoxemia and a high rate of fatality even now, roughly 25–30 %, which develops under various pathogenic conditions [1]. Lung injury is triggered by inhalation of airborne causative agents, designated as direct lung injury, and develops in the context of systemic disorders such as sepsis, designated as indirect lung injury [2]. Direct lung injury has been reported to occur during infection with severe acute respiratory syndrome (SARS) virus [3] or H5N1, a highly pathogenic avian influenza virus [4, 5]. The serum levels of interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and IL-6 were

Published online: 14 February 2013

elevated in the cases of fatal human H5N1 influenza virus infection [6], and several cytokines, including TNF-α, IL-1β, IL-6, IL-8, and IL-10 showed strikingly increased concentrations in the bronchoalveolar lavage (BAL) fluids of these patients [7–9].

In animal models of direct lung injury induced by intratracheal administration of lipopolysaccharide (LPS), acute and robust influx of inflammatory cells into the lungs is observed but these inflammatory changes are completely resolved within 48 h, without demonstration of either alveolar epithelial injury or vascular leakage, typical histopathological features of ARDS [10, 11]. Recently, we reported an animal model of fulminant ARDS established by sensitizing mice with α-galactosylceramide (α-GalCer), a potent activator of natural killer T (NKT) cells, followed by a challenge with LPS [12]. In this model, all mice rapidly died with diffuse alveolar damage in the lungs, which was accompanied by a striking increase of IFN-γ and TNF-α concentrations in BAL fluids [12].

A variety of proinflammatory cytokines and chemokines, such as TNF-α, IL-1β, IL-6, and IL-8, are reported to play critical roles in the pathogenic mechanism of ARDS [7–9], whereas lower production of antiinflammatory mediators such as IL-10 and IL-1 receptor antagonist is associated with the generally poor prognosis [13]. Based on an understanding of the involvement of these cytokines, various antiinflammatory agents, including corticosteroids [14, 15], statins [16], prostaglandin E1 [17], neutrophil elastase inhibitors [18], and inhibitors of arachidonic acid metabolism [19, 20], have been tested for their clinical efficacy in ARDS therapy. However, a truly efficacious agent has not yet been established.

Type I IFN, discovered as an endogenously synthesized antiviral protein [21], is deeply involved in acute and chronic inflammatory responses, including delayed-type hypersensitivity [22], experimental autoimmune encephalomyelitis [23], and collagen-induced arthritis [24]. In addition to these nonantiviral actions, this cytokine inhibits division of certain tumor cell lines [25], promotes cytolytic activity of NK cells and T cells [26], and enhances expression of MHC molecules [27]. Thus, type I IFN has been gradually recognized as an important player in the cytokine network that leads to regulation of innate and acquired immune responses [21]. In clinical settings, type I IFN is used for the treatment of viral hepatitis and multiple sclerosis [21]. Recently, in animal experiments, antiinflammatory effects as a result of type I IFN administration have been reported in experimental autoimmune encephalomyelitis [28], rheumatoid arthritis [29], bacterial peritonitis [30], and lung inflammation [31].

With this background in mind, the present study addressed how type I IFNs affect the clinical course and inflammatory responses in our mouse model of fulminant ARDS sensitized with α-GalCer and challenged with LPS. We found that low-dose administration of IFN-α improved survival and attenuated inflammatory responses in fulminant ARDS mice and that these effects were associated with suppression of IFN-γ synthesis in the lung.

MATERIALS AND METHODS

Animals

C57BL/6 mice were bred in a pathogen-free environment at the Institute for Animal Experimentation, Tohoku University Graduate School of Medicine. All mice were used for experiments at 7–8 weeks of age. All experimental protocols described in the present study were approved by the Ethics Review Committee for Animal Experimentation of Tohoku University.

Reagents

α-GalCer, purchased from Funakoshi (Tokyo, Japan), was dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA) at 5 mg/ml and then diluted with phosphate-buffered saline (PBS) to a final dose of 0.4 % DMSO for *in vivo* use. LPS from *Escherichia coli* (O111: B4) was purchased from Sigma-Aldrich.

Fulminant ARDS Model

To induce lung injury, mice were anesthetized by intraperitoneal injection of 70 mg/kg of pentobarbital (Abbott Laboratories, North Chicago, IL, USA) and then restrained on a small board and 50 μl of α-GalCer (1 μg) was injected into each mouse by inserting a 24-gauge intravenous catheter (Terumo, Tokyo, Japan) into the trachea. Twenty-four hours later, 50 μl of LPS (50 μg) was administered via the same route. Sham-operated mice were injected with 0.4 % DMSO-containing PBS (dPBS) into the trachea and 24 h later, 50 μl of PBS was administered via the same route.

Treatment with IFN-α

Recombinant human IFN-αA/D was provided by the Nippon Roche Research Center (Kamakura, Japan). Mice were administered various amounts of IFN-α intranasally at various time intervals after LPS injection.

IFN-α was administrated intratracheally with LPS at the same time only at 0 h.

Wet/Dry Lung Weight Ratio

The lungs of mice treated with IFN-α or PBS were weighed 48 h after LPS challenge to determine the final wet lung weight. They were then dried in an oven at 60 °C for 48 h and weighed again to determine the dry weight. The wet/dry (W/D) weight ratio was then calculated.

Histological Examination

The lung specimens obtained from mice were fixed in 10 % buffered formalin, dehydrated, and embedded in paraffin. Sections were cut and stained with hematoxylin–eosin (HE) stains using standard staining procedures at the Biomedical Research Core, the Animal Pathology Platform of Tohoku University Graduate School of Medicine. We measured histological evidence of tissue injury using the Lung Injury Scoring System recommended by the American Thoracic Society (ATS) [32]. Lung Injury Scoring System parameters include neutrophils in the alveolar space, neutrophils in the interstitial space, hyaline membranes, proteinaceous debris filling the airspaces, and alveolar septal thickening. At least 20 random high-power fields (400× total magnification) were independently scored in a blinded fashion. Each of five histological findings was graded using a three-tiered scheme that is summarized in Table 1. The sum of each of the five independent variables shown in Table 1 was weighted according to the relevance ascribed to each feature by the ATS Committee and then was normalized to the number of fields evaluated.

Table 1. Lung Injury Scoring System

	Score per field		
Parameters	0	1	2
A. Neutrophils in the alveolar space	None	1–5	>5
B. Neutrophils in the interstitial space	None	1–5	>5
C. Hyaline membranes	None	1	>1
D. Proteinaceous debris filling the airspaces	None	1	>1
E. Alveolar septal thickening	<2×	2×–4×	>4×

Score = $[(20 \times A) + (14 \times B) + (7 \times C) + (7 \times D) + (2 \times E)]$ / (number of fields × 100). Reference: American Thoracic Society [34]

Preparation of BAL Fluids and Lung Homogenates

Mice were sacrificed at 6 h after LPS exposure. Their chests were opened, their tracheae were cannulated (22 G I.V. catheter), and 1 ml of PBS was infused intratracheally and withdrawn. This procedure was performed three times. BAL fluids were stored at −80 °C until cytokines were measured. After collecting BAL fluids, pulmonary circulation was rinsed by injection of 3 ml PBS into the right ventricle. Lungs were then harvested and stored in 1.5 ml of PBS. The lungs in PBS were later homogenized and centrifuged at 1,600 rpm for 10 min at 4 °C, and the supernatants were stored at −80 °C until cytokines were measured.

Measurement of Cytokine Concentrations

The concentrations of IFN-γ and TNF-α in the supernatants of lung homogenates and BAL fluids were measured by ELISA using capture and biotinylated developing antibodies (BD Biosciences, Franklin Lakes, NJ, USA). The detection limits were 15 and 5 pg/ml, respectively.

Statistical Analysis

Analysis of data was conducted using JMP Pro® 9.0.2 software (SAS Institute Inc., Cary, NC, USA) on a Macintosh computer. Data are expressed as mean ± SD. Statistical analysis between groups was performed using analysis of variance with a *post hoc* analysis (Fisher's protected least significant difference test). Survival data were analyzed using the logrank test. A p value <0.05 was considered significant.

RESULTS

Effect of IFN-α on the Survival of Fulminant ARDS Mice

In our earlier studies [12], intratracheal administration of LPS following sensitization with α-GalCer led to the development of fulminant ARDS in mice, which caused fatal outcomes within 72 h after LPS injection. In the current study, we investigated whether IFN-α affected the clinical course of fulminant ARDS mice. For this purpose, mice were treated with various doses of IFN-α (100 IU, 1,000 IU, and 10,000 IU/mouse) or with PBS as a vehicle control 12 h before, simultaneously, and 24 h, 48 h, and 72 h after LPS injection. As shown in Fig. 1, all mice treated with PBS died within

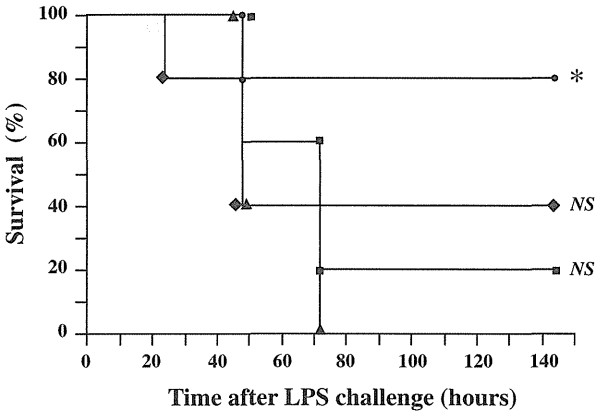

Fig. 1. Effect of IFN-α treatment on the survival of fulminant ARDS mice. Mice received intratracheal instillation of LPS 24 h after administration of α-GalCer via the same route. These mice were treated with various doses of IFN-α or PBS as a control 12 h before (intranasally: i.n.), simultaneously (intratracheally), and 24 h (i.n.), 48 h (i.n.) and 72 h (i.n.) after LPS injection. The number of live mice was counted every 24 h after the LPS challenge. Each group consisted of five mice. *Circles* IFN-α 100 IU, *squares* IFN-α 1,000 IU, *diamonds* IFN-α 10,000 IU, *triangles* PBS. *NS* not significant; *$P<0.05$ compared with PBS-treated mice.

72 h, whereas treatment with low-dose IFN-α, 100 IU/mouse, significantly prolonged the survival duration of fulminant ARDS mice. However, higher doses of IFN-α (1,000 IU and 10,000 IU/mouse) did not prolong survival. These results demonstrated that low-dose, but not high-dose, IFN-α protected mice from death caused by fulminant ARDS.

Effect of Low-dose IFN-α Treatment on Lung Injury in Fulminant ARDS Mice

Next, we addressed the effect of IFN-α administration on lung injuries developed in fulminant ARDS mice by conducting histopathological analyses. Using the results illustrated in Fig. 1 as a basis, α-GalCer-sensitized and LPS-challenged mice were treated with low-dose IFN-α (100 IU/mouse) or PBS, and lung sections stained with HE were analyzed under a microscope 72 h after LPS challenge. As shown in Fig. 2, massive infiltration of neutrophils not only into the intraalveolar septa but also into the alveolar spaces was observed in the α-GalCer-sensitized and LPS-challenged mice that received PBS treatment, whereas these inflammatory responses were strikingly attenuated when mice were treated with low-dose IFN-α. For an objective evaluation of the inflammatory changes, we quantified the histological changes of the tissue injuries using a Lung Injury Scoring System. As

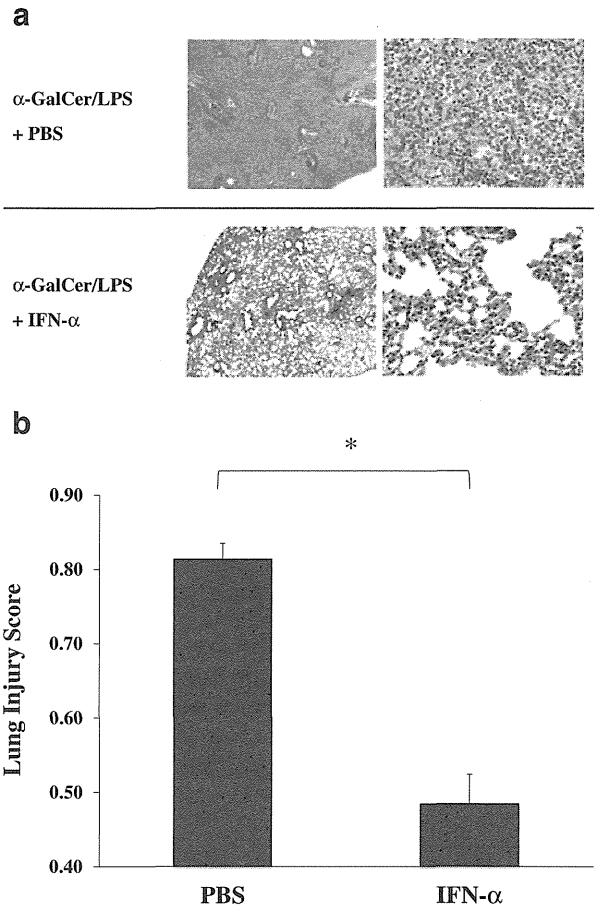

Fig. 2. Effect of low-dose IFN-α treatment on histological changes in fulminant ARDS mice. Mice received intratracheal instillation of LPS 24 h after administration of α-GalCer via the same route (α-GalCer/LPS). These mice were treated with IFN-α (100 IU) or PBS 12 h before (intranasally: i.n.), simultaneously (intratracheally), and 24 h (i.n.) and 48 h (i.n.) after LPS injection. Sections of lungs 72 h after LPS challenge were stained with hematoxylin and eosin and observed under a light microscope. *Representative pictures* of three mice are shown at magnifications of ×40 (*left*) and ×400 (*right*).

shown in Fig. 2b, the lung injury scores were significantly lower in low-dose IFN-α-treated mice than those in control mice. These results indicate that low-dose IFN-α effectively inhibited the development of FARDS caused by α-GalCer sensitization and LPS challenge.

In addition, to evaluate the degree of lung edema, a characteristic of ARDS, we examined W/D lung weight ratios 48 h after LPS challenge. As Fig. 3 shows, the W/D lung weight ratios were significantly lower in mice treated with IFN-α than in PBS-treated mice, and the levels in the former mice were almost equivalent to

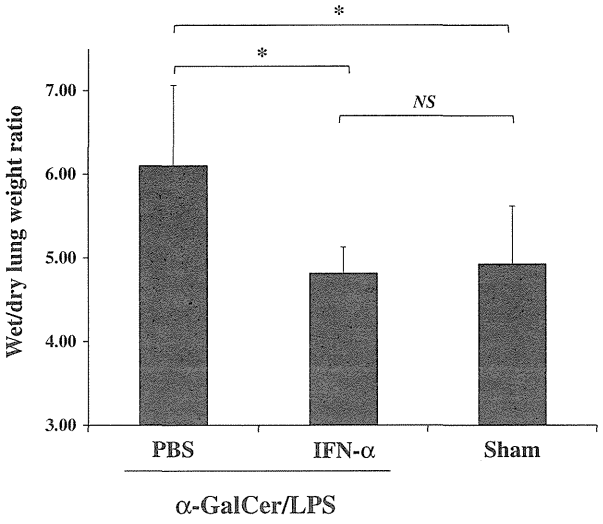

Fig. 3. Effect of low-dose IFN-α treatment on lung edema in fulminant ARDS mice. Mice received intratracheal instillation of LPS 24 h after administration of α-GalCer via the same route (α-GalCer/LPS). In a sham group, mice received intratracheal instillation of PBS 24 h after administration of PBS via the same route (sham). The wet and dry lung weight ratio was examined 48 h after challenge injection of LPS or PBS. Each *column* represents the mean ± SD of five mice. *NS* not significant; $*P<0.05$.

those in sham-operated mice. These results clearly demonstrated that low-dose IFN-α treatment mitigated the development of fulminant ARDS by attenuating inflammatory responses in the lung.

Effect of Low-dose IFN-α Treatment on Cytokine Synthesis in the Lungs

To further investigate the mechanism for the beneficial effects of IFN-α on fulminant ARDS, we evaluated the synthesis in the lungs of IFN-γ and TNF-α, both of which were reported as critical cytokines in our mouse model of fulminant ARDS [12]. For this purpose, concentrations of these cytokines in BAL fluids and lung homogenates were measured 6 h after LPS challenge. As shown in Fig. 4a, treatment with low-dose IFN-α significantly reduced the IFN-γ levels in both BAL fluids and lung homogenates in α-GalCer-sensitized and LPS-challenged mice, compared to those in PBS-treated mice, and the IFN-γ levels in the lung homogenates of IFN-α-treated mice were almost as low as those in sham-operated mice. TNF-α synthesis in the lung, as determined by assay of BAL fluids and lung homogenates, was also augmented in mice sensitized with α-GalCer and challenged with LPS. In striking contrast to the observed impact upon IFN-γ levels, low-dose IFN-α treatment did not reduce the synthesis of TNF-α in the lung, measured by assaying the two types of lung specimens (Fig. 4b).

DISCUSSION

In the present study, we demonstrated that (1) administration of low-dose IFN-α prolonged the survival of fulminant ARDS mice sensitized with α-GalCer and challenged with LPS, but high dose IFN-α did not provide this benefit; (2) low-dose IFN-α treatment attenuated the diffuse alveolar damage that occurs as a consequence of massive infiltration of neutrophils, a typical histological feature of ARDS, and it reduced the W/D lung weight ratio, an indicator of lung edema, compared to that in the PBS-treated group; and (3) low-dose IFN-α treatment led to decreased production of IFN-γ, but not of TNF-α, according to assays of BAL fluids and lung homogenates in fulminant ARDS mice. These results indicate that low-dose IFN-α has an antiinflammatory and therapeutic effect in a mouse model of fulminant ARDS and suggest that reduced production of IFN-γ in the lungs may be involved in the beneficial effects of this treatment.

Infection with H5N1, a highly pathogenic avian influenza virus, is reported to cause fulminant ARDS with histopathologically defined diffuse alveolar damage in the lung, which is often refractory even to advanced medical therapy [4, 5]. This pathogenic condition is associated not only with viral replication in bronchial epithelial cells but also with uncontrolled production of inflammatory cytokines, the so-called "cytokine storm" in the lungs that is associated with a fatal outcome in many cases [4, 32]. Thus, control of excessive inflammatory responses may provide an effective therapeutic target against fulminant ARDS caused by the H5N1 influenza virus. Type I IFNs are synthesized and secreted by virus-infected cells, which upregulate the expression of antiviral genes and render the host resistant to this infection [21]. Recently, Haasbach *et al.* demonstrated that low-dose type I IFN treatment was effective in protecting mice from infection with H5N1 influenza virus [33]. Though this effect is thought to be due to the antiviral activity of type I IFN, it remains unanswered whether this treatment leads to suppression of the inflammatory response at the infected sites. In the present study, on the other hand, we examined the effect of type I IFN treatment on the fulminant ARDS developed by a

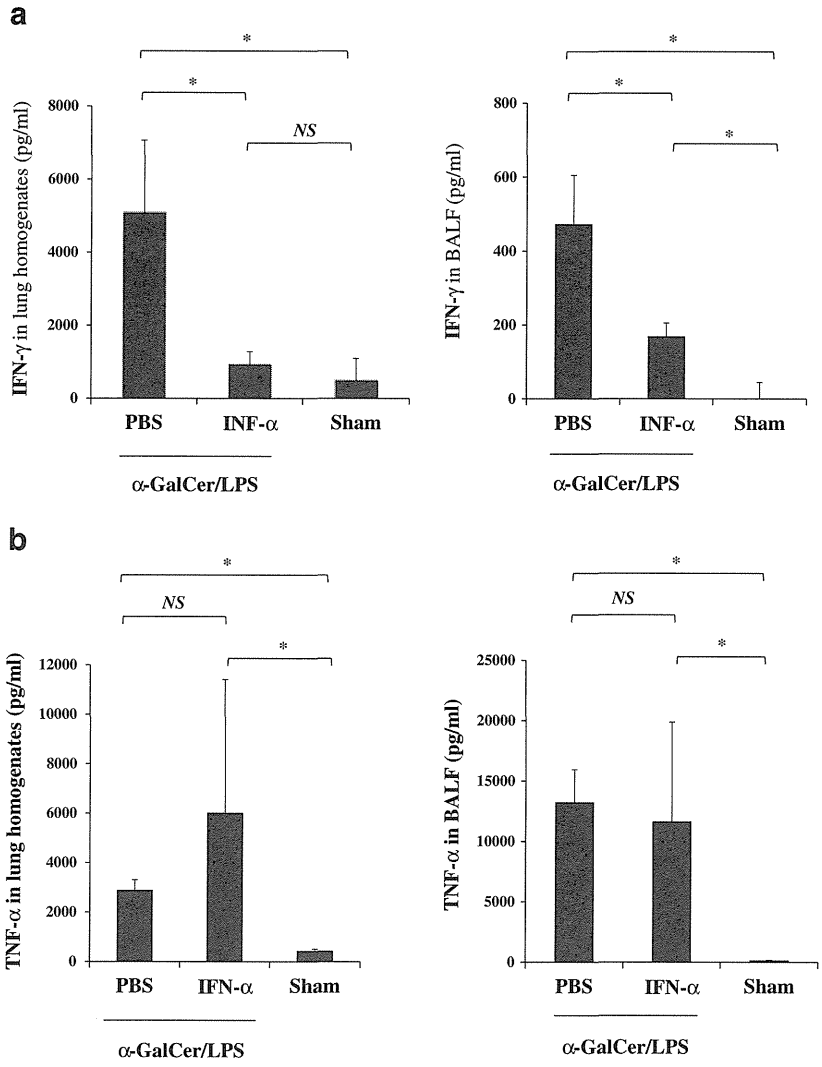

Fig. 4. Effect of low-dose IFN-α treatment on cytokine synthesis in fulminant ARDS mice. Mice received intratracheal instillation of LPS 24 h after administration of α-GalCer via the same route (α-GalCer/LPS). In a sham group, mice received intratracheal instillation of PBS 24 h after administration of PBS via the same route (sham). These mice were treated with IFN-α (100 IU) or PBS 12 h before (intranasally: i.n.) and simultaneously (intratracheally) after LPS injection. The concentrations of IFN-γ and TNF-α in lung homogenates and BAL fluids were measured 6 h after challenge injection of LPS or PBS. Each *column* represents the mean ± SD of four or five mice. *NS* not significant; $*P<0.05$.

mechanism unrelated to viral infection, which may aid in an understanding of its therapeutic potential via an antiinflammatory effect separate from antiviral activity, although it remains unclear if our model is valid for analyzing the pathogenic mechanism of fulminant ARDS caused by influenza virus infection and other clinically important conditions.

In animal models of bacterial peritonitis [30] and acute lung injury associated with subarachnoid hemorrhage [31], high-dose IFN-β was used to demonstrate antiinflammatory effects. However, the present study showed that low-dose IFN-α was effective in extending the survival time of fulminant ARDS mice. The antiinflammatory effects of lower doses of IFN-α, as recognized in our current study, have been reported in previous investigations in various animal models of inflammatory diseases [33–37]. In *in vitro* experiments by Amadori *et al.* [38], IFN-α was shown to suppress the expression of a TNF-α gene by swine alveolar macrophages at dosages as low as 0.5 IU/ml. The same

investigators also reported that orally administered IFN-α suppressed the expression of inflammatory cytokines such as IFN-γ, TNF-α, and IL-6 after weaning in piglets [39]. In contrast, in cattle, parenteral administration of high-dose, but not low-dose IFN-α, actually promoted inflammatory responses [40]. Taken together with these previous studies, our present findings suggest that low-dose administration of IFN-α may exert antiinflammatory effects by inhibiting the production of proinflammatory cytokines, although the precise mechanism remains to be elucidated.

Several mechanisms have been proposed to explain the antiinflammatory effects of type I IFN. In previous studies by Quin *et al.*, interaction of IFN-β with its receptor was shown to trigger the induction of suppressor of cytokine signaling 1 (SOCS1), which then regulated IFN-γ synthesis by inhibiting the signal transducer and activator of transcription (STAT1α) [41]. Other investigators have also reported that the antiinflammatory effects of type I IFNs are mediated by the production of IL-10 [42] and by suppressing TNF-α synthesis through the generation of tristetraprolin (TTP), an antiinflammatory protein [43]. In our study, the antiinflammatory effects of IFN-α were not associated with alteration of TNF-α synthesis, unlike the remarkable reduction of IFN-γ synthesis, and IL-10 was not detected in the lungs of mice in any of the groups (data not shown). In agreement with these results, we previously reported on the critical role of IFN-γ, rather than TNF-α, which role was limited, in the pathogenesis of fulminant ARDS developed in α-GalCer-sensitized and LPS-challenged mice. In this model, NKT cells and Gr-1^+ monocytes were involved in the synthesis of IFN-γ [12]. These findings raise the possibility that IFN-α may ameliorate the effects of fulminant ARDS by accelerating SOCS1 activation, which leads to the suppression of IFN-γ synthesis by NKT cells and Gr-1^+ monocytes. However, in the present study, attempts to address the precise mechanism in the beneficial effect of IFN-α remain to be made, and further investigation is needed to clarify this possibility.

In conclusion, we demonstrated that low-dose IFN-α improved the survival of fulminant ARDS mice that were sensitized with α-GalCer and challenged with LPS by suppressing the production of IFN-γ and subsequent inflammatory responses. These results suggest that this cytokine could be a possible therapeutic option for ARDS, which is refractory even to advanced medical therapies currently available in clinical settings. In general, higher doses of type I IFNs are administered to patients with hepatitis virus infection and multiple sclerosis [44–46]. However, higher dose treatments are known to be associated with various adverse effects such as chills, fever, muscle aches, headaches, and depression. In this respect, less frequent adverse effects might be expected as an additional benefit of lower dose IFN-α treatment. Thus, the present study may provide a significant impetus for further development of promising ARDS treatments.

ACKNOWLEDGMENTS

This study was supported by a grant from the Ministry of Education, Culture, Sports, Science, and Technology (Grant-in-Aid for Challenging Exploratory Research (23659841)) and a grant from the Ministry of Health, Labor, and Welfare of Japan (Research on Emerging and Re-emerging Infectious Diseases; 22-SHINKOU-IPPAN-014).

Conflict of interest None.

REFERENCES

1. Wheeler, A.P., G.R. Bernard, B.T. Thompson, D. Schoenfeld, H.P. Wiedemann, B. de Boisblanc, A.F. Connors Jr., R.D. Hite, and A.L. Harabin. 2006. Pulmonary-artery versus central venous catheter to guide treatment of acute lung injury. *The New England Journal of Medicine* 354: 2213–2224.
2. Ware, L.B., and M.A. Matthay. 2000. The acute respiratory distress syndrome. *The New England Journal of Medicine* 342: 1334–1349.
3. Lee, N., D. Hui, A. Wu, P. Chan, P. Cameron, G.M. Joynt, A. Ahuja, M.Y. Yung, C.B. Leung, K.F. To, S.F. Lui, C.C. Szeto, S. Chung, and J.J. Sung. 2003. A major outbreak of severe acute respiratory syndrome in Hong Kong. *The New England Journal of Medicine* 348: 1986–1994.
4. Beigel, J.H., J. Farrar, A.M. Han, F.G. Hayden, R. Hyer, M.D. de Jong, S. Lochindarat, T.K. Nguyen, T.H. Nguyen, T.H. Tran, A. Nicoll, S. Touch, and K.Y. Yuen. 2005. Avian influenza A (H5N1) infection in humans. *The New England Journal of Medicine* 353: 1374–1385.
5. Kawachi, S., S.T. Luong, M. Shigematsu, H. Furuya, T.T. Phung, P.H. Phan, H. Nunoi, L.T. Nguyen, and K. Suzuki. 2009. Risk parameters of fulminant acute respiratory distress syndrome and avian influenza (H5N1) infection in Vietnamese children. *Journal of Infectious Diseases* 200: 510–515.
6. To, K.F., P.K. Chan, K.F. Chan, W.K. Lee, W.Y. Lam, K.F. Wong, N.L. Tang, D.N. Tsang, R.Y. Sung, T.A. Buckley, J.S. Tam, and A.F. Cheng. 2001. Pathology of fatal human infection associated with avian influenza A H5N1 virus. *Journal of Medical Virology* 63: 242–246.
7. Bhatia, M., and S. Moochhala. 2004. Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *The Journal of Pathology* 202: 145–156.

8. Park, W.Y., R.B. Goodman, K.P. Steinberg, J.T. Ruzinski, F. Radella 2nd, D.R. Park, J. Pugin, S.J. Skerrett, L.D. Hudson, and T.R. Martin. 2001. Cytokine balance in the lungs of patients with acute respiratory distress syndrome. *American Journal of Respiratory and Critical Care Medicine* 164: 1896–1903.
9. Shimabukuro, D.W., T. Sawa, and M.A. Gropper. 2003. Injury and repair in lung and airways. *Critical Care Medicine* 31: S524–S531.
10. Bastarache, J.A., and T.S. Blackwell. 2009. Development of animal models for the acute respiratory distress syndrome. *Disease Models & Mechanisms* 2: 218–223.
11. Matute-Bello, G., C.W. Frevert, and T.R. Martin. 2008. Animal models of acute lung injury. *American Journal of Physiology. Lung Cellular and Molecular Physiology* 295: L379–L399.
12. Aoyagi, T., N. Yamamoto, M. Hatta, D. Tanno, A. Miyazato, K. Ishii, K. Suzuki, T. Nakayama, M. Taniguchi, H. Kunishima, Y. Hirakata, M. Kaku, and K. Kawakami. 2011. Activation of pulmonary invariant NKT cells leads to exacerbation of acute lung injury caused by LPS through local production of IFN-γ and TNF-α by Gr-1+ monocytes. *International Immunology* 23: 97–108.
13. Donnelly, S.C., R.M. Strieter, P.T. Reid, S.L. Kunkel, M.D. Burdick, I. Armstrong, A. Mackenzie, and C. Haslett. 1996. The association between mortality rates and decreased concentrations of interleukin-10 and interleukin-1 receptor antagonist in the lung fluids of patients with the adult respiratory distress syndrome. *Annals of Internal Medicine* 125: 191–196.
14. Peter, J.V., P. John, P.L. Graham, J.L. Moran, I.A. George, and A. Bersten. 2008. Corticosteroids in the prevention and treatment of acute respiratory distress syndrome (ARDS) in adults: meta-analysis. *BMJ* 336: 1006–1009.
15. Tang, B.M., J.C. Craig, G.D. Eslick, I. Seppelt, and A.S. McLean. 2009. Use of corticosteroids in acute lung injury and acute respiratory distress syndrome: a systematic review and meta-analysis. *Critical Care Medicine* 37: 1594–1603.
16. Craig, T.R., M.J. Duffy, M. Shyamsundar, C. McDowell, C.M. O'Kane, J.S. Elborn, and D.F. McAuley. 2011. A randomized clinical trial of hydroxymethylglutaryl-coenzyme a reductase inhibition for acute lung injury (The HARP Study). *American Journal of Respiratory and Critical Care Medicine* 183: 620–626.
17. Bone, R.C., G. Slotman, R. Maunder, H. Silverman, T.M. Hyers, M.D. Kerstein, and J.J. Ursprung. 1989. Randomized double-blind, multicenter study of prostaglandin E1 in patients with the adult respiratory distress syndrome. Prostaglandin E1 Study Group. *Chest* 96: 114–119.
18. Zeiher, B.G., A. Artigas, J.L. Vincent, A. Dmitrienko, K. Jackson, B.T. Thompson, and G. Bernard. 2004. Neutrophil elastase inhibition in acute lung injury: results of the STRIVE study. *Critical Care Medicine* 32: 1695–1702.
19. 2000. Ketoconazole for early treatment of acute lung injury and acute respiratory distress syndrome: a randomized controlled trial. The ARDS Network. *Journal of the American Medical Association* 283:1995–2002.
20. Bernard, G.R., A.P. Wheeler, J.A. Russell, R. Schein, W.R. Summer, K.P. Steinberg, W.J. Fulkerson, P.E. Wright, B.W. Christman, W.D. Dupont, S.B. Higgins, and B.B. Swindell. 1997. The effects of ibuprofen on the physiology and survival of patients with sepsis. The Ibuprofen in Sepsis Study Group. *The New England Journal of Medicine* 336: 912–918.
21. Billiau, A. 2006. Anti-inflammatory properties of type I interferons. *Antiviral Research* 71: 108–116.
22. De Maeyer, E., J. De Maeyer-Guignard, and M. Vandeputte. 1975. Inhibition by interferon of delayed-type hypersensitivity in the mouse. *Proceedings of the National Academy of Sciences of the United States of America* 72: 1753–1757.
23. Abreu, S.L. 1982. Suppression of experimental allergic encephalomyelitis by interferon. *Immunological Communications* 11: 1–7.
24. Triantaphyllopoulos, K.A., R.O. Williams, H. Tailor, and Y. Chernajovsky. 1999. Amelioration of collagen-induced arthritis and suppression of interferon-gamma, interleukin-12, and tumor necrosis factor alpha production by interferon-beta gene therapy. *Arthritis and Rheumatism* 42: 90–99.
25. Uno, K., S. Shimizu, K. Inaba, M. Kitaura, K. Nakahira, T. Kato, Y. Yamaguchi, and S. Muramatsu. 1988. Effect of recombinant human interferon-alpha A/D on *in vivo* murine tumor cell growth. *Cancer Research* 48: 2366–2371.
26. Moore, M., W.J. White, and M.R. Potter. 1980. Modulation of target cell susceptibility to human natural killer cells by interferon. *International Journal of Cancer* 25: 565–572.
27. Ransohoff, R.M., C. Devajyothi, M.L. Estes, G. Babcock, R.A. Rudick, E.M. Frohman, and B.P. Barna. 1991. Interferon-beta specifically inhibits interferon-gamma-induced class II major histocompatibility complex gene transcription in a human astrocytoma cell line. *Journal of Neuroimmunology* 33: 103–112.
28. Billiau, A., H. Heremans, F. Vandekerckhove, R. Dijkmans, H. Sobis, E. Meulepas, and H. Carton. 1988. Enhancement of experimental allergic encephalomyelitis in mice by antibodies against IFN-gamma. *Journal of Immunology* 140: 1506–1510.
29. van Holten, J., K. Reedquist, P. Sattonet-Roche, T.J. Smeets, C. Plater-Zyberk, M.J. Vervoordeldonk, and P.P. Tak. 2004. Treatment with recombinant interferon-beta reduces inflammation and slows cartilage destruction in the collagen-induced arthritis model of rheumatoid arthritis. *Arthritis Research & Therapy* 6: R239–R249.
30. Weighardt, H., S. Kaiser-Moore, S. Schlautkotter, T. Rossmann-Bloeck, U. Schleicher, C. Bogdan, and B. Holzmann. 2006. Type I IFN modulates host defense and late hyperinflammation in septic peritonitis. *Journal of Immunology* 177: 5623–5630.
31. Cobelens, P.M., I.A. Tiebosch, R.M. Dijkhuizen, P.H. van der Meide, R. Zwartbol, C.J. Heijnen, J. Kesecioglu, and W.M. van den Bergh. 2010. Interferon-β attenuates lung inflammation following experimental subarachnoid hemorrhage. *Critical Care* 14: R157.
32. Matute-Bello, G., G. Downey, B.B. Moore, *et al.* 2011. An official American Thoracic Society workshop report: Features and measurements of experimental acute lung injury in animals. *American Journal of Respiratory Cell and Molecular Biology* 44: 725–738.
33. Liem, N.T., N. Nakajima, P. Phat le, Y. Sato, H.N. Thach, P.V. Hung, L.T. San, H. Katano, T. Kumasaka, T. Oka, S. Kawachi, T. Matsushita, T. Sata, K. Kudo, and K. Suzuki. 2008. H5N1-infected cells in lung with diffuse alveolar damage in exudative phase from a fatal case in Vietnam. *Japanese Journal of Infectious Diseases* 61: 157–160.
34. Haasbach, E., K. Droebner, A.B. Vogel, and O. Planz. 2011. Low-dose interferon type I treatment is effective against H5N1 and swine-origin H1N1 influenza A viruses *in vitro* and *in vivo*. *Journal of Interferon and Cytokine Research* 31: 515–525.
35. Beilharz, M.W., J.M. Cummins, and A.L. Bennett. 2007. Protection from lethal influenza virus challenge by oral type 1 interferon. *Biochemical and Biophysical Research Communications* 355: 740–744.
36. Cummins, M.J., A. Papas, G.M. Kammer, and P.C. Fox. 2003. Treatment of primary Sjogren's syndrome with low-dose human interferon alfa administered by the oromucosal route: combined phase III results. *Arthritis and Rheumatism* 49: 585–593.
37. Ito, A., E. Isogai, K. Yoshioka, K. Sato, N. Himeno, and T. Gotanda. 2010. Ability of orally administered IFN-α4 to inhibit naturally occurring gingival inflammation in dogs. *Journal of Veterinary Medical Science* 72: 1145–1151.
38. Ohya, K., T. Matsumura, N. Itchoda, K. Ohashi, M. Onuma, and C. Sugimoto. 2005. Ability of orally administered IFN-alpha-containing transgenic potato extracts to inhibit Listeria monocytogenes infection. *Journal of Interferon and Cytokine Research* 25: 459–466.

39. Begni, B., M. Amadori, M. Ritelli, and D. Podavini. 2005. Effects of IFN-alpha on the inflammatory response of swine leukocytes to bacterial endotoxin. *Journal of Interferon and Cytokine Research* 25: 202–208.
40. Amadori, M., M. Farinacci, B. Begni, R. Faita, D. Podavini, and M. Colitti. 2009. Effects of interferon-α on the inflammatory response of swine peripheral blood mononuclear cells. *Journal of Interferon and Cytokine Research* 29: 241–247.
41. Straub, O.C. 1995. Studies on the suitability of alpha-hybrid interferon application in cattle. *Comparative Immunology, Microbiology and Infectious Diseases* 18: 239–243.
42. Qin, H., C.A. Wilson, S.J. Lee, and E.N. Benveniste. 2006. IFN-beta-induced SOCS-1 negatively regulates CD40 gene expression in macrophages and microglia. *The FASEB Journal* 20: 985–987.
43. Chang, E.Y., B. Guo, S.E. Doyle, and G. Cheng. 2007. Cutting edge: Involvement of the type I IFN production and signaling pathway in lipopolysaccharide-induced IL-10 production. *Journal of Immunology* 178: 6705–6709.
44. Sauer, I., B. Schaljo, C. Vogl, I. Gattermeier, T. Kolbe, M. Muller, P.J. Blackshear, and P. Kovarik. 2006. Interferons limit inflammatory responses by induction of tristetraprolin. *Blood* 107: 4790–4797.
45. Cooksley, W.G. 2004. The role of interferon therapy in hepatitis B. *MedGenMed* 6: 16.
46. Pereira, A.A., and I.M. Jacobson. 2009. New and experimental therapies for HCV. *Nature Reviews. Gastroenterology & Hepatology* 6: 403–411.

MODERN PATHOLOGY (2012), 1–13

Pathological study of archival lung tissues from five fatal cases of avian H5N1 influenza in Vietnam

Noriko Nakajima[1], Ngo Van Tin[2], Yuko Sato[1], Hoang Ngoc Thach[2], Harutaka Katano[1], Pho Hong Diep[2], Toshio Kumasaka[3], Nguyen Trung Thuy[2], Hideki Hasegawa[1], Luong Thi San[4], Shoji Kawachi[5], Nguyen Thanh Liem[4], Kazuo Suzuki[6] and Tetsutaro Sata[1,7]

[1]*Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan;* [2]*Department of Anatomical Pathology, National Hospital of Pediatrics, Hanoi, Vietnam;* [3]*Department of Pathology, Japanese Red Cross Medical Center, Tokyo, Japan;* [4]*National Hospital of Pediatrics, Hanoi, Vietnam;* [5]*Surgical Operation Department, National Center for Global Health and Medicine, Tokyo, Japan;* [6]*Safety Control Department, University Hospital, School of Medicine, and General Medical Education Center, Teikyo University, Tokyo, Japan and* [7]*Toyama Institute of Health, Toyama, Japan*

Highly pathogenic avian H5N1 influenza virus (H5N1) infection in humans causes acute respiratory distress syndrome, leading to multiple organ failure. Five fatal cases of H5N1 infection in Vietnam were analyzed pathologically to reveal virus distribution, and local proinflammatory cytokine and chemokine expression profiles in formalin-fixed, paraffin-embedded lung tissues. Our main histopathological findings showed diffuse alveolar damage in the lungs. The infiltration of myeloperoxidase-positive and/or CD68 (clone KP-1)-positive neutrophils and monocytes/macrophages was remarkable in the alveolar septa and alveolar spaces. Immunohistochemistry revealed that H5N1 mainly infected alveolar epithelial cells and monocytes/macrophages in lungs. H5N1 replication was confirmed by detecting H5N1 mRNA in epithelial cells using *in situ* hybridization. Quantitation of H5N1 RNA using quantitative reverse transcription PCR assays revealed that the level of H5N1 RNA was increased in cases during early phases of the disease. We quantified the expression of tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-8, regulated on activation normal T-cell expressed and secreted (commonly known as RANTES), and interferon-gamma-inducible protein of 10 kDa (IP-10) in formalin-fixed, paraffin-embedded lung sections. Their expression levels correlated with H5N1 RNA copy numbers detected in the same lung region. Double immunofluorescence staining revealed that TNF-α, IL-6, IL-8 and IP-10 were expressed in epithelial cells and/or monocytes/macrophages. In particular, IL-6 was also expressed in endothelial cells. The dissemination of H5N1 beyond respiratory organs was not confirmed in two cases examined in this study.
Modern Pathology advance online publication, 23 November 2012; doi:10.1038/modpathol.2012.193

Keywords: autopsy; cytokine; chemokine; H5N1; influenza; immunohistochemistry; lung

Since the outbreak of highly pathogenic avian H5N1 influenza virus infection in humans in 2003, the WHO has reported 608 confirmed cases from 15 countries, with a mortality rate of about 60% by August 2012 (http://www.who.int/influenza/human_animal_interface/EN_GIP_20120810CumulativeNumberH5N1cases.pdf). The potential for mutation and reassortment of the viral genome, which may be responsible for human-to-human transmission, has increased the threat of an influenza pandemic.

Many H5N1-infected patients develop acute respiratory distress syndrome and die because of respiratory failure or multiple organ failure.[1,2] Pathological study of autopsied cases revealed that the H5N1 virus infected type I and type II pneumocytes and caused primary viral pneumonia, which further developed into acute respiratory distress syndrome.[3–18] Although seasonal influenza virus infection is also associated with pneumonia, the virus mainly infects epithelial cells of the upper respiratory tract.[19–21] Complications involving

Correspondence: Dr N Nakajima, MD, PhD, Department of Pathology, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku, Tokyo 162-8640, Japan.
E-mail: tenko@nih.go.jp
Received 22 June 2012; revised 20 September 2012; accepted 20 September 2012; published online 23 November 2012