プログラム

11 月 14日(金)

はじめに 13:00-13:20

・あいさつ

林 憲一(厚生労働省医薬食品局血液対策課企画官)

・安全なガンマグロブリン製剤開発—人工ガンマグロブリンの実用化をめざして—

鈴木和男(千葉大院医)

1. 人工化の開発動向と現状 13:20-13:40

座長:高橋 啓(東邦大医療センター)

1-1 人工ポリクローナル免疫グロブリン

鈴木和男、古谷昌弘、大野尚仁、荒谷康昭、亀岡洋祐、長尾朋和、高橋 啓

(千葉大院医、積水化学工業、東京薬科大学、横浜市大、医薬基盤研、東邦大医療センター)

2. IVIg 治療の動向 13:40-15:00

座長:湯村和子(自治医科大学)

2-1 MPO−ANCA陽性の顕微鏡的多発血管炎のIVIg治療

—急速進行性糸球体腎炎を伴う顕微鏡的多発血管炎に向けて—

武會恵理(北野病院)

2-1-A 追加発言:抵抗性への有効性

山縣邦弘(筑波大院医)

2-2 急性期川崎病血管炎へのガンマグロブリン超大量単回数投与の有用性とその不応例への治療選択

佐地 勉(東邦大学医療センター)

休 憩 15:00-15:20

3. In vitro 評価系 15:20-15:40

座長:平橋淳一(東大病院)

3-1 In vitro での評価系の確立と作用機序解析へのアプローチ

大野尚仁 、三浦典子、長尾朋和、宇野賀津子、 常 賀、 鈴木和男

(東京薬科大学、千葉大院医、ルイパスツール医学研)

4. 血管炎の疫学調査　15:40–16:20

座長：鈴木和男

4–1 疫学調査と国際会議 EUVAS/EULAR/ACR の会議報告

藤元昭一、小林茂人、鈴木和男（宮崎大医、順天堂越谷病院、千葉大学院医）

4–2 血管炎の概念・分類・診断基準の相違について

–EULAR/ACR の分類基準、診断基準、疾患名の動向

小林茂人（順天堂越谷病院）

4–3 2009 年–ANCA–Workshop の最新動向

鈴木和男（千葉大院医）

5. トピックス　16:20–17:50

座長：有村義宏（杏林大学）

・血管炎の基礎および臨床研究の先端研究者によるトピックス

5–1 小型血管炎と悪性腫瘍—本邦報告例の検討—

有村義宏、軽部美穂、山田　明（杏林大学第一内科）

5–2 Ig 融合蛋白による自己免疫性心筋炎の治療

—新しい Ig 融合蛋白による治療を目指して—

塙　晴雄、相澤義房（新潟大学大学院医歯学総合研究科）

5–3 大量γグロブリン（IVIG）が著効した MPO－ANCA 関連腎炎の一例

長澤康行、今井圓裕（大阪大学大学院）

5–4 IVIg 療法の小児劇症型 ARDS（FARDS）に対する効果について

–ハノイ国立小児病院の症例から–

河内正治[1,2]、布井博幸[4]、鈴木和男[2,3]

（[1]国立国際医療センター　手術部麻酔科・ICU、[2]国立感染症研究所　免疫部

[3]千葉大学大学院　医学研究院感染分子生物学・炎症制御学、[4]宮崎大学医学部

生殖発達医学講座小児科学分野・小児科学）

5–5 In Vitro における IVIg 治療の作用機序解析

常　賀、長尾朋和、中山俊憲*、鈴木和男

（千葉大学大学院医学研究院　免疫発生学・炎症制御学、*免疫発生学）

6. コメントとまとめ

橋本博史（順天堂大学・医）、直江史郎（桐蔭横浜大学）、岡崎富男（呉共済病院）

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Toshiko Ito-Ihara, Eri Muso, Shigeto Kobayashi, Kazuko Uno, Naoto Tamura, Yuji Yamanishi, Atsushi Fukatsu, Richard A. Watts, David G.I. Scott, David R.W. Jayne, Kazuo Suzuki, Hiroshi	A comparative study of the diagnostic accuracy of ELISA systems for the detection of anti-neutrophil cytoplasm antibodies available in Japan and Europe.	Clin. Exp. Rheumatol	in press		2008
Shigeto Kobayashi, Akihiko Ito, Daisuke Okuzaki, Hiroaki Onda, Norikazu Yabuta, Ippei Nagamori , Kazuo Suzuki, Hiroshi Hashimoto and Hiroshi Nojima.	Expression profiling of PBMC-based diagnostic gene markers isolated from vasculitis patients.	DNA Research	15	253-265	2008
Akiyoshi Hoshino, Tomokazu Nagao, Noriko Nagi-Miura, Naohito Ohno, Masato Yasuhara, Kenji Yamamoto, Toshinori Nakayama, Kazuo Suzuki.	MPO-ANCA induces IL-17 production by activated neutrophils in vitro via classical complement pathway-dependent manner.	J. Autoimmunity	31	79-89	2008
Xiao G, Miyazato A, Inden K, Nakamura K, Shiratori K, Nakagawa K, Miyazawa T, Suzuki K, Kaku M, Kawakami K.	Cryptococcus neoformans inhibits nitric oxide synthesis caused by CpG-oligodeoxynucleotide-stimulated macrophages in a fashion independent of capsular	Microbiol Immunol.	52	171-179	2008
Nakamura K, Miyazato A, Xiao G, Hatta M, Inden K, Aoyagi T, Shiratori K, Takeda K, Akira S, Saijo S, Iwakura Y, Adachi Y, Ohno N, Suzuki K, Fujita J, Kaku M, Kawakami K.	Deoxynucleic acids from Cryptococcus neoformans activate myeloid dendritic cells via a TLR9-dependent pathway.	J Immunol.	15	4067-1074	2008
Watts RA, Scott DGI, Jayne DRW, Ito-Ihara T, Muso E, Fujimoto S, Harabuchi Y, Kobayashi S, Suzuki K, Hashimoto H	Renal vasculitis in Japan and the UK - are there differences in epidemiology and clinical phenotype?	Nephrolo Dial Transplant	23	3928-3931	2008
Joh K, Muso E, Shigematsu H, Nose M, Nagata M, Arimura Y, Yumura W, Wada T, Nitta K, Makino H, Taguma Y, Kaneoka H, Suzuki Y, Kobayashi M, Koyama A, Usui J, Ozaki S, Tomino Y, Hashimoto H, Yamagata;	Renal pathology of ANCA-related vasculitis: Proposal for standardization of pathological diagnosis in Japan	Clin Exp Nephrol	12	277-291	2008
古宮俊幸、辻井知美、石床学、田原佐知子、米本智美、塚本達雄、武曾恵理	血液透析導入時にヘパリン起因性血小版減少症をきたした関節リウマチの2症例	透析会誌	41(9)	627-633	2008
武曾恵理	ガンマプログリン大量療法の血管炎に対する効果 特集 血管炎の診療に役立つ新たな知見	リウマチ科	40(1)	44-52	2008

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
Watanabe R, Hanawa H, Yoshida T, Ito M, Isoda M, Chang H, Toba K, Yoshida K, Kojima M, Otaki K, Ding L, Hao K, Kato K, Kodama M, Aizawa Y.	Gene expression profiles of cardiomyocytes in rat autoimmune myocarditis by DNA microarray and increase of regenerating gene family.	Transl Res.	152	119-127	2008
Chang H, Hanawa H, Yoshida T, Hayashi M, Liu H, Ding L, Otaki K, Hao K, Yoshida K, Kato K, Toba K, Kodama M, Maruyama H, Miyazaki J, Aizawa Y.	Alteration of IL-17 related protein expressions in experimental autoimmune myocarditis and inhibition of IL-17 by IL-10-Ig fusion gene transfer.	Circ J.	72	813-819.	2008
Makiyama Y, Toba K, Kato K, Hirono S, Ozawa T, Saigawa T, Minagawa S, Isoda M, Asami F, Ikarashi N, Oda M, Moriyama M, Higashimura M, Kitajima T, Otaki K	Imatinib mesilate inhibits neointimal hyperplasia via growth inhibition of vascular smooth muscle cells in a rat model of balloon injury.	Tohoku J Exp Med.	215	299-306	2008
Ozawa T, Kato K, Toba K, Oda M, Isoda M, Asami F, Ikarashi N, Yanagawa T, Moriyama M, Higashimura M, Kitajima T, Otaki K, Takayama T, Hirono S, Okura Y, Hanawa H,	Serum erythropoietin level as a marker of limb ischemia.	Int J Cardiol.	130	106-8	2008
Thandavarayan RA, Watanabe K, Ma M, Veeraveedu PT, Gurusamy N, Palaniyandi SS, Zhang S, Muslin AJ, Kodama M, Aizawa Y.	14-3-3 protein regulates Ask1 signaling and protects against diabetic cardiomyopathy.	Biochem Pharmacol.	75	1797-806.	2008
Watanabe R, Hanawa H, Yoshida T, Ito M, Isoda M, Chang H, Toba K, Yoshida K, Kojima M, Otaki K, Ding L, Hao K, Kato K, Kodama M, Aizawa Y.	Gene expression profiles of cardiomyocytes in rat autoimmune myocarditis by DNA microarray and increase of regenerating gene family.	Transl Res.	152	119-27	2008
Muro T, Maruyama Y, Onishi K, Saze M, Pkada E, Matsuura H, Saji T	Mimicking Kawasaki disease in burned children : Report of four cases	Burns	In Press	In Press	In Press

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
Onouchi Y, Gunji T, Burns JC, Shimizu C, Newburger W, Yashiro M, Nakamura Y, Yanagawa H, Wakui K, Fukushima Y, Kishi F, Hamamoto K, Terai M, Sato Y, Ouchi K, Saji T, Nariai A, Kaburagi Y, Yoshikawa T, Suzuki K, Tanaka T, Nagai T, Cho H, Fujino A, Sekine A, Nakamichi R, Tsunoda T, Kawasaki T, Nakamura Y, Hata A	ITPKC functional polymorphism associated with Kawasaki disease susceptibility and formation of coronary artery aneurysms.	Nature Genetics	40(1)	35-42	2008
Tougan T, Oda A, Okuzaki D, Kobayashi S, Hasimoto H, Nojima H	Focused microarray analysis of peripheral mononuclear blood cells from Churg-Strauss syndrome patients.	DNA Research	15(2):	:103-14.	2008
Mamegano K, Kuroki K, Miyashita R, Kusaoi K, Kobayashi S, Matsuta K, K Maenaka K, Colonna M, Ozaki S, Hashimoto H, Takasaki T, Tokunaga K, Tsuchiya N	Association of LILRA2 (IL1,LIR7) splice site polymorphism with systemic lupus erythematosus and microscopic polyangiitis	Genes and Immunity	10, F9	:214-223	2008
Seta N, Tajima M, Kobayashi S, Kawakami Y, Hashimoto H, Kuwana K	Autoreactive T cell responses to myeloperoxidase in patients with antineutrophil cytoplasmic antibody-associateed vasculitis and healthy individuals.	Mod Rheumatol	18(6):	593-600	2008
Kobayashi S, Ito A, Okuzaki D, Onda H, Yabuta N, Nagamori I, Suzuki K, Hashimoto H, Nojima H.	Expression profiling of PBMC-based diagnostic gene markers isolated from vasculitis patients.	DNA Res	15(4)	253-65.	2008
小林茂人、木田一成	ANCA関連血管炎に関する疫学、血管炎の診療に役立つ新たな知見	リウマチ科	40(2)	136-143	2008
Hirotsugu Iwatani, Yasuyuki Nagasawa, Kazumasa Oka, Yoshitaka Isaka, Enyu Imai	Valvular injury in a patient with PR3-ANCA-associated glomerulonephritis	Nat Clin Practice Nephrol	4	576-582	2008
湯村和子	臨床医学の展望2008 腎臓病学3 ANCA関連腎炎.	日本医事新報	4373	66	2008
湯村和子	慢性腎臓病と高齢者の腎障害.	日本老年医学会雑誌	45(1)	1-8	2008

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
Nagai Y, Itabashi M, Mizutani M, Ogawa T, Yumura W, Tsuchiya K, Nitta K	○A case report of uncompensated alkalosis induced by daily plasmapheresis in a patient with thrombotic thrombocytopenic purpura	Ther Apher Dial	12(1)	86-90	2008
Oishi T, Iida A, Otsubo S, Kamatani Y, Usami M, Takaei T, Uchida K, Tsuchiya K, Saito S, Ohnishi Y, Tokunaga K, Nitta K, Kawaguchi Y, Kamatani N, Kochi Y, Shimane K, Yamamoto K, Nakamura Y, Yumura W, Matsuda K	A functional SNP in the NKK2.5-binding site of ITPR3 promoter is associated with susceptibility to systemic lupus erythematosus in Japanese population.	J Hum Genet	53	151-162	2008
伊藤千春, 湯村和子	ANCA関連血管炎の評価法においてBVASの意義と問題点.	リウマチ科	40 (1)	17-25	2008
Kamatani Y, Matsuda K, Oishi T, Otsubo S, Yamazaki K, Iida A, Hosono N, Kubo M, Yumura W, NittaK, Katagiri T, Kawaguchi Y, Kamatani N, Nakamura Y	Indentification of a significant association of a single nucleotide polymorphism in TNXB with systemic lupus erythematosus in Japanes population.	J Hum Genet	53	64-73	2008
戸澤亮子, 湯村和子	パラプロテイン腎症	腎と透析	64(6)	958-962	2008
板橋美津世, 湯村和子, 塚田三佐緒, 代田さつき, 武井卓, 小川哲也, 芳田 工, 内田啓子, 土谷 健, 新田孝作	MPO-ANCA関連血管炎の臨床病理学的アプローチによる腎病態の解析	日本腎臓学会誌	50(7)	927-933	2008
Hirayama K, Yamagata K, Kobayashi M, Koyama A	Anti-glomerular basement membrane antibody disease in Japan: part of the nationwide rapidly progressive glomerulonephritis survey in Japan.	Clin Exp Nephrol	12(5),	339-347	2008
山縣邦弘、臼井丈一	難治性Wegenerにおけるリツキシマブの効果	リウマチ科	40(1)	53-57	2008
山縣邦弘、臼井丈一	腎・尿路系疾患 急速進行性腎炎	綜合臨床	5 増刊	1257-1259	2008
藤元昭一	透析導入を避けられますか？M.P.ワンポイントアドバイス。	Medical Practice	25	535	2008
藤元昭一	RPGN、ANCA関連血管炎の疫学。特集 急速進行性糸球体腎炎	日腎会誌	51(2)	in press	2009
Tada R, Nagi-Miura N, Adachi Y, Ohno N	The influence of culture conditions on vasculitis and anaphylactoid shock induced by fungal pathogen Candida albicans cell wall extract in mice	Microb Pathog	44	379-388	2008

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
Nagi-Miura N, Adachi Y, Ohno N.	Coronary arteritis induced by CAWS (Candida albicans water-soluble fraction) in various strains of mice	Nippon Ishinkin Gakkai Zasshi	49	287-292	2008
Harada T, Miura NN, Adachi Y, Nakajima M, Yadomae T, Ohno N.	Highly expressed dectin-1 on bone marrow-derived dendritic cells regulates the sensitivity to beta-glucan in DBA/2 mice	J Interferon Cytokine Res	28	477-486	2008
大原関利章、横内 幸、高橋 啓	:川崎病の最近の病因論、	小児内科	41	21-25	2009
高橋 啓、大原関利章、横内 幸	冠状動脈病変の病理、	小児内科	41	30-33	2009
大原関利章、横内 幸、伊原 文恵、若山 恵、高橋 啓	急性期川崎病血管炎の病理	東邦医会誌	55	129-131	2008
Rowley AH, Baker SC, Shulman ST, Garcia FL, Fox LM, Kos IM, Crawford SE, Russo PA, Hammadeh R, Takahashi K, Orenstein JM:	RNA-containing cytoplasmic inclusion bodies in ciliated bronchial epithelium months to years after acute Kawasaki disease.	PLoS ONE.	13;3	e1582.	2008
大原関利章、横内 幸、高橋 啓	:川崎病の最近の病因論、	小児内科	41	21-25	2009
高橋 啓、大原関利章、横内 幸	冠状動脈病変の病理、	小児内科	41	30-33	2009
荒谷康昭	好中球機能異常による呼吸器不全—ミエロペルオキシダーゼ欠損を中心に—	医学のあゆみ	224	861-862	2008
荒谷康昭	真菌・細菌感染防御の鍵:好中球Myeloperoxidase。細胞	細胞	41	56-59	2009
Uno K, Matsuzaki T, Yagi K, Hamuro J, Okuno K	Pre-operative Intracellular Peripheral Monocyte Glutathione Levels Correlate with anti-Tumor Immune-responses and Predict Overall Survival of Patients with Colorectal Carcinoma	Cytokine	Vol.43, 2008	289	2008
Uno K, Tominaga M, Hasegawa G, Fukui M, Yagi K, Tanigawa M, Fujita S, Yoshikawa T, Nakamura N	Multiple Cytokine/Chemokine Analysis Related to the Development of Impaired Glucose Metabolism and DM in Human Plasma	Ann Nutr Metab	Vol.53	61	2008
宇野賀津子、武曾恵理、猪原登志子、八木克己、鈴木和男	MPO-ANCA腎炎のサイトカイン・ケモカイン動態	PASKEN JOURNAL	Vol.20 ,2007	1-5	2008
宇野賀津子、富永真澄、長谷川剛二、福井道明、八木克己、谷川真理、藤田晢也、吉川敏一、中村直登	境界型糖尿病から糖尿病への進展をサイトカイン・ケモカイン動態:多項目同時測定法による網羅的解析	PASKEN JOURNAL	Vol.20 ,2007	6-11	2008
宇野賀津子、富永真澄、長谷川剛二、福井道明、八木克己、藤田晢也、吉川敏一、中村直登	2型糖尿病の発症過程におけるインターフェロン—α産生能の変化	PASKEN JOURNAL	Vol.20 ,2007	12-18	2008

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
安田ゐう子、下田泰治、宇野賀津子、立石成人、八木克己、古谷誠一、鈴木和男、藤田晢也	マウスの免疫学的特性と1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP)感受性	PASKEN JOURNAL	Vol.20 ,2007	19-28	2008
安田ゐう子、下田泰治、宇野賀津子、立石成人、古谷誠一、藤田晢也	一過性前脳虚血再開通後海馬CA1におけるミクログリアの動態とNG2の発現	PASKEN JOURNAL	Vol.20 ,2007	29-41	2008
Yasuda Y, Shimoda T, Uno K, Tateishi N, Furuya S, Yagi K, Suzuki K, Fujita S.	The effects of MPTP on the activation of microglia/astrocytes and cytokine/chemokine levels in different mice strains.	J Neuroimmunol.	204(1-2)	43-51	2008
Osada N, Hashimoto K, Kameoka Y, Hirata M, Tanuma R, Uno Y, Inoue I,Hida M, Suzuki Y, Sugano S, Terao K, Kusuda J, Takahashi I.	Large-scale analysis of Macaca fascicularis transcripts and inference of genetic divergence between M. fascicularis and M. mulatta.	BMC Genomics	9	90	2008
Tougan T, Onda H, Okuzaki D, Kobayashi S, Hashimoto H, Nojima H.	Focused microarray analysis of peripheral mononuclear blood cells from Churg-Strauss syndrome patients.	DNA Research	15(2)	103-14	2008
Tougan T, Okuzaki D, Nojima H.	Chum-RNA allows preparation of a high-quality cDNA library from a single-cell quantity of mRNA without PCR amplification.	Nuc. Acids Res.	36(15)	e92	2008
Utomo A, Hirahashi J*, Mekala D, Ziffle JV, Xiao L, Saffaripour S, Wagner DD, Shapiro SD, Lowell C, Mayadas TN. *the 1st co-	Requirement for Vav proteins in post-recruitment neutrophil cytotoxicity in IgG but not complement C3-dependent injury.	J Immunol	180	6279-6287	2008

書籍

著者氏名	論文タイトル名	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
鈴木和男	感染症を抑え込め：大規模予測モデル「感染症の脅威：パンデミックへの備えは万全か」		日経サイエンス別冊163	日経サイエンス社	東京	2008年11月	38-46
鈴木和男	シミュレーションによる感染症の対策支援「感染症の脅威:パンデミックへの備えは万全か」		日経サイエンス別冊163	日経サイエンス社	東京	2008年11月	38-46
鈴木和男	人工ガンマグロブリン製剤の開発の現状		ファルマシア	日本薬学会	東京	2009年1月	45: 17-22
長尾朋和　鈴木和男	ANCAをめぐる基礎的研究の進歩		呼吸器科	化学評論社	東京	2008年11月	14:348-354
鈴木和男	特集―感染症防御・慢性疾患の初期機構―総論「好中球の機能」		細胞	ニューサイエンス社	東京	2009年2月	41: 48-50
長尾朋和　鈴木和男	「好中球機能研究の最前線―新たな展開―」好中球機能異常による血管炎・腎炎		細胞	ニューサイエンス社	東京	2009年2月	41: 60-63
田村直人、小林茂人	抗リン脂質抗体症候群,	井村裕夫	わかりやすい内科学	文光堂	東京	2008	423～426
小林茂人	関節リウマチ	富野康日己、望月正隆	疾患と薬物治療：知っておきたいcommon disease 101	医歯薬出版株式会社	東京	2008	399－401
小林茂人、木田一成	多発関節炎、耳の痛みを訴えてきた42歳の女性	橋本博史	New専門医を目指すケース・メソッド・アプローチ，膠原病・リウマチ	日本医事新報社	東京	2008	305-315
小林茂人	リウマチ専門医試験　例題と解説	日本リウマチ学会	リウマチ専門医試験　例題と解説	メディカルビュー社	東京	2008	不明
湯村和子	全身性エイトマトーデスによる腎障害	山口徹、北原光夫、福井次矢	今日の治療指針2008	医学書院	東京	2008	453-456

別 刷 り 一 覧

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Watts RA, Scott DGI, Jayne DRW, Ito-Ihara T, Muso E, Fujimoto S, Harabuchi Y, Kobayashi S, Suzuki K, Hashimoto H	Renal vasculitis in Japan and the UK - are there differences in epidemiology and clinical phenotype?	Nephrolo Dial Transplant	23	3928-3931	2008
Nakamura K, Miyazato A, Xiao G, Hatta M, Inden K, Aoyagi T, Shiratori K, Takeda K, Akira S, Saijo S, Iwakura Y, Adachi Y, Ohno N, Suzuki K, Fujita J, Kaku M, Kawakami K.	Deoxynucleic acids from Cryptococcus neoformans activate myeloid dendritic cells via a TLR9-dependent pathway.	J Immunol.	15	4067-1074	2008
Shigeto Kobayashi, Akihiko Ito, Daisuke Okuzaki, Hiroaki Onda, Norikazu Yabuta, Ippei Nagamori , Kazuo Suzuki, Hiroshi Hashimoto and Hiroshi Nojima.	Expression profiling of PBMC-based diagnostic gene markers isolated from vasculitis patients.	DNA Research	15	253-265	2008
Akiyoshi Hoshino, Tomokazu Nagao, Noriko Nagi-Miura, Naohito Ohno, Masato Yasuhara, Kenji Yamamoto, Toshinori Nakayama, Kazuo Suzuki.	MPO-ANCA induces IL-17 production by activated neutrophils in vitro via classical complement pathway-dependent manner.	J. Autoimmunity	31	79-89	2008
古宮俊幸、辻井知美、石床学、田原佐知子、米本智美、塚本達雄、武曾恵理	血液透析導入時にヘパリン起因性血小版減少症をきたした関節リウマチの2症例	透析会誌	41 (9)	627-633	2008
Joh K, Muso E, Shigematsu H, Nose M, Nagata M, Arimura Y, Yumura W, Wada T, Nitta K, Makino H, Taguma Y, Kaneoka H, Suzuki Y, Kobayashi M, Koyama A, Usui J, Ozaki S, Tomino Y, Hashimoto H, Yamagata:	Renal pathology of ANCA-related vasculitis: Proposal for standardization of pathological diagnosis in Japan	Clin Exp Nephrol	12	277-291	2008
Chang H, Hanawa H, Yoshida T, Hayashi M, Liu H, Ding L, Otaki K, Hao K, Yoshida K, Kato K, Toba K, Kodama M, Maruyama H, Miyazaki J, Aizawa Y.	Alteration of IL-17 related protein expressions in experimental autoimmune myocarditis and inhibition of IL-17 by IL-10-Ig fusion gene transfer.	Circ J.	72	813-819.	2008
Muro T, Maruyama Y, Onishi K, Saze M, Pkada E, Matsuura H, Saji T	Mimicking Kawasaki disease in burned children : Report of four cases	Burns	In Press	In Press	In Press
Tougan T, Onda H, Okuzaki D, Kobayashi S, Hashimoto H, Nojima H.	Focused microarray analysis of peripheral mononuclear blood cells from Churg-Strauss syndrome patients.	DNA Research	15(2)	103-14	2008
小林茂人、木田一成	ANCA関連血管炎に関する疫学、血管炎の診療に役立つ新たな知見	リウマチ科	40(2)	136-143	2008

別 刷 り 一 覧

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Hirotsugu Iwatani, Yasuyuki Nagasawa, Kazumasa Oka, Yoshitaka Isaka, Enyu Imai	Valvular injury in a patient with PR3-ANCA-associated glomerulonephritis	Nat Clin Practice Nephrol	4	576-582	2008
伊藤千春, 湯村和子	ANCA関連血管炎の評価法においてBVASの意義と問題点.	リウマチ科	40 (1)	17-25	2008
Kamatani Y, Matsuda K, Oishi T, Otsubo S, Yamazaki K, Iida A, Hosono N, Kubo M, Yumura W, NittaK, Katagiri T, Kawaguchi Y, Kamatani N, Nakamura Y	Indentification of a significant association of a single nucleotide polymorphism in TNXB with systemic lupus erythematosus in Japanes population.	J Hum Genet	53	64-73	2008
板橋美津世, 湯村和子, 塚田三佐緒, 代田さつき, 武井卓, 小川哲也, 芳田 工, 内田啓子, 土谷 健, 新田孝作	MPO-ANCA関連血管炎の臨床病理学的アプローチによる腎病態の解析	日本腎臓学会誌	50(7)	927-933	2008
Hirayama K, Yamagata K, Kobayashi M, Koyama A	Anti-glomerular basement membrane antibody disease in Japan: part of the nationwide rapidly progressive glomerulonephritis survey in	Clin Exp Nephrol	12(5),	339-347	2008
Nagi-Miura N, Adachi Y, Ohno N.	Coronary arteritis induced by CAWS (Candida albicans water-soluble fraction) in various strains of mice	Nippon Ishinkin Gakkai Zasshi	49	287-292	2008
Harada T, Miura NN, Adachi Y, Nakajima M, Yadomae T, Ohno N.	Highly expressed dectin-1 on bone marrow-derived dendritic cells regulates the sensitivity to beta-glucan in DBA/2 mice	J Interferon Cytokine Res	28	477-486	2008
Rowley AH, Baker SC, Shulman ST, Garcia FL, Fox LM, Kos IM, Crawford SE, Russo PA, Hammadeh R, Takahashi K, Orenstein JM:	RNA-containing cytoplasmic inclusion bodies in ciliated bronchial epithelium months to years after acute Kawasaki disease.	PLoS ONE.	13;3	e1582.	2008
大原関利章、横内 幸、伊原文恵、若山 恵、高橋 啓	急性期川崎病血管炎の病理	東邦医会誌	55	129-131	2008
荒谷康昭	好中球機能異常による呼吸器不全—ミエロペルオキシダーゼ欠損を中心に—	医学のあゆみ	224	861-862	2008
荒谷康昭	真菌・細菌感染防御の鍵：好中球Myeloperoxidase。細胞	細胞	41	56-59	2009
Yasuda Y, Shimoda T, Uno K, Tateishi N, Furuya S, Yagi K, Suzuki K, Fujita S.	The effects of MPTP on the activation of microglia/astrocytes and cytokine/chemokine levels in different mice strains.	J Neuroimmunol	204(1-2)	43-51	2008
Utomo A, Hirahashi J*, Mekala D, Ziffle JV, Xiao L, Saffaripour S, Wagner DD, Shapiro SD, Lowell C, Mayadas TN. *the 1st co-	Requirement for Vav proteins in post-recruitment neutrophil cytotoxicity in IgG but not complement C3-dependent injury.	J Immunol	180	6279-6287	2008

Nephrol Dial Transplant (2008) 23: 3928–3931
doi: 10.1093/ndt/gfn354
Advance Access publication 19 June 2008

Original Article

NDT
Nephrology Dialysis Transplantation

Renal vasculitis in Japan and the UK—are there differences in epidemiology and clinical phenotype?

Richard A. Watts[1], David G. I. Scott[2], David R. W. Jayne[3], Toshiko Ito-Ihara[4,5], Eri Muso[6], Shouichi Fujimoto[7], Yasuki Harabuchi[8], Shigeto Kobayashi[9], Kazuo Suzuki[10,11] and Hiroshi Hashimoto[9]

[1]School of Medicine, Health Policy and Practice, University of East Anglia, [2]Department of Rheumatology, Norfolk and Norwich University Hospital, Norwich, [3]Lupus and Vasculitis Clinic, Addenbrookes' Hospital, Cambridge, UK, [4]Department of Nephrology and Cardiovascular Medicine, Louis Pasteur Centre for Medical Research, Graduate School of Medicine, Kyoto University, Kyoto, Japan, [5]Department of Clinical Rheumatology, Musculoskeletal Research Group, School of Clinical Medical Sciences, Newcastle University, Newcastle upon Tyne, UK, [6]Department of Nephrology and Dialysis, Kitano Hospital, Tazuke Kofukai Medical Research Institute, Osaka, [7]First Department of Internal Medicine, Miyazaki Medical College, University of Miyazaki, Miyazaki, [8]Department of Otolaryngology, Asahikawa Medical College, Asahikawa, [9]Department of Internal Medicine and Rheumatology, Juntendo University, School of Medicine, [10]National Institute of Infectious Diseases (NIID-NIH), Tokyo and [11]Inflammation Program, Department of Immunology, Chiba University Graduate School of Medicine, Chiba, Japan

Abstract

Background. The epidemiology of renal vasculitis in different populations is poorly understood. A recent study from Japan suggests that whilst the overall incidence is similar to that reported from Europe, the clinical phenotype is different, with Wegener's granulomatosis being very much less common. The aim of this study was to compare the incidence of renal vasculitis in the UK with recent data from a Japanese population.

Methods. Incident patients with renal vasculitis were identified prospectively between 2000 and 2004 from a well-defined UK population. The case notes were reviewed and clinical features extracted. Classification between Wegener's granulomatosis, microscopic polyangiitis and Churg Strauss syndrome was performed using a predetermined algorithm. Inclusion criteria were (i) new patients with vasculitis with or without histological confirmation, (ii) renal involvement and (iii) positive serology for anti-neutrophil cytoplasmic antibody (ANCA).

Results. We identified 27 cases of renal vasculitis (Wegener's granulomatosis 13, microscopic polyangiitis 11, Churg Strauss syndrome 3) fulfilling the case definition. The overall average age was 63.5 years which is less than those of the Japanese patients. The overall annual incidence of renal vasculitis was 12.2/million similar to Japan. The annual incidence of Wegener's granulomatosis was 5.8/million, microscopic polyangiitis 4.9/million and Churg Strauss syndrome 1.4/million. ENT and neurological involvement were much less common in Japan. No patients with cANCA/PR3 were seen in Japan. Wegener's granulomatosis seems to be much less common in Japan than the UK.

Discussion. Whilst the overall occurrence of renal vasculitis is similar in Japan to the UK, the clinical phenotype is very different with microscopic polyangiitis predominating in Japan.

Keywords: ANCA; epidemiology; microscopic polyangiitis; vasculitis; Wegener's granulomatosis

Introduction

The ANCA-associated vasculitides (AAV) are a group of rare conditions characterized by the occurrence of systemic necrotising vasculitis and the presence of anti-neutrophil cytoplasmic antibody (ANCA) in serum. Over the past 20 years there have been several studies describing the epidemiology in European and American populations. There is a broad consensus that in these populations, (i) the overall annual incidence is ~10–20/million, (ii) the peak age of onset is 65–74 years and (iii) they are very rare in childhood [1]. Comparing the incidence and prevalence in different regions of Europe suggests that there are some potentially significant differences in the various subtypes. A comparative study between groups in Norway, England and Spain in which care was taken to try to classify/define the patients in a consistent fashion showed that Wegener's granulomatosis (WG) was more common in North Norway with an annual incidence of 10.5/million compared with Spain (4.9/million), whilst microscopic polyangiitis (MPA) was more common in Spain (11.6/million) than Norway

Correspondence and offprint requests to: Richard Watts, School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, NR4 7TJ, UK. Tel: +44-160-3593-570; Fax: +44-160-3593-570; E-mail: richard.watts@uea.ac.uk

© The Author [2008]. Published by Oxford University Press on behalf of ERA-EDTA. All rights reserved.
For Permissions, please e-mail: journals.permissions@oxfordjournals.org

(2.7/million) [2]. There is little data available from outside Europe. Reports from Japan suggest that the majority of patients with AAV are classified as MPA and that MPO-ANCA-associated disease is much more common than PR3-ANCA-associated disease [3]. A recent study of renal vasculitis during 2000–2004 from Miyazaki prefecture in Japan reported an incidence of MPA of 14.8/million [4]. This study was unable to find any patients with WG or Churg Strauss syndrome (CSS). Since 1988 we have maintained a prospective register of all patients presenting with AAV in the Norwich area. A previous study from this cohort reported an annual incidence of renal vasculitis of 18/million in the 1990s using a different case definition [5]. The aim of the present study was to compare the incidence of renal vasculitis in the UK with Japan during the period 2000–2004 using the same case definitions and investigate whether there are differences in disease expression between the two populations.

Methods

UK population

We have since 1988 maintained a prospective register of patients with systemic vasculitis (NORVASC) who attend the Norfolk and Norwich University Hospital (NNUH), which is the single central referral centre for a stable and ethnically homogenous population of ~500 000. The study area covers a geographically isolated coastal region in Eastern England allowing the population to be well defined and therefore suitable for epidemiological studies over a prolonged period of time [6]. We have previously described our methods of case ascertainment [7].

All patients attending the NNUH as outpatients, day admissions and hospitalized with a new clinical diagnosis of systemic vasculitis between 1 January 2000 and 31 December 2004 were identified. The computerized records of the histopathology department were searched for patients with a histological appearance on a renal biopsy consistent with renal vasculitis. The case notes were reviewed retrospectively to identify all patients with renal vasculitis either at presentation or developing subsequently. Patients fulfilling the inclusion criteria for the Miyazaki study were identified: (i) patients with a new diagnosis of WG, MPA, CSS or RLV, with or without histological confirmation, between 1 January 2000 and 31 December 2004; (ii) renal involvement with or without other organ involvement attributable to active WG, MPA, CSS or RLV (necrotizing vasculitis and pauci-immune necrotizing, crescentic glomerulonephritis); and (iii) positive serology for ANCA [4]. A negative ANCA was accepted if there was histological evidence of vasculitis.

The recently described European consensus classification algorithm for the classification of vasculitis was used to classify patients [8]. This uses an algorithm to classify vasculitis and utilizes the ACR (1990) criteria and the Chapel Hill Consensus Conference definitions. Using this approach renal limited vasculitis is placed with MPA.

The denominator population was the same as used in previous studies—patients registered with general practitioners in the former Norwich Health Authority [6]. The estimated 2002 population is 445 000 (215 000 males). The population is ~95% Caucasian of UK descent, which is lower than the average for England (Watts 2004). Around 9% of the population is aged >75 years, which is higher than the average for England [7]. The gender balance is similar to the UK as a whole.

Age- and gender-specific incidence rates were calculated using the number of incident cases as the numerator and the population as the denominator. Confidence intervals (95%) were calculated using the Poisson distribution. Comparisons between the UK and Japanese studies were made using the chi-square test.

ANCA was determined by indirect immunofluorescence and by ELISA for PR3/MPO specificity using commercial available kits.

The Norwich Local Research Ethics Committee approved the study.

Results

We identified 36 cases of AAV during 2000–2004. There were 27 cases of renal vasculitis (WG = 13, MPA = 11, CSS = 3) fulfilling the case definition. The overall average age was 63.5 years (WG = 63.9, MPA = 65.4, CSS = 54.4), which is less than those of the Japanese patients. The overall annual incidence of renal vasculitis was 12.2/million. The annual incidence of WG was 5.8/million, MPA 4.9/million and CSS 1.4/million. Comparison with the Japanese study is shown in Table 1. The most striking differences are that no patients with WG or CSS were seen in the Japanese study. There were also significant differences in the pattern of ANCA positivity seen. Over 90% of the Japanese patients were pANCA/MPO positive whereas 55% of the UK patients were pANCA/MPO positive. No Japanese patients were found to be cANCA or PR3-ANCA positive. Five patients in Japan were ANCA negative and two in the UK, both in the MPA group. There were also striking differences in the clinical features especially ENT and neurological

Table 1. Comparison epidemiology and clinical features of renal vasculitis in UK and Japan

	Japan	UK	
Male:female	24:32	13:14	
Mean age (year)	70.4	63.5	
Incidence total/million	14.8 (10.8–18.9)	12.2 (8.0–17.7)	
Incidence MPA/million	14.8 (10.8–18.9)	5.0 (2.4–8.8)	
Incidence WG/million	0	5.8 (2.9–9.4)	
Incidence CSS/million	0	1.4 (0.3–3.9)	
Feature	Japan (%)	UK (%)	
ENT	1 (1.8)	18 (66.6)	$P < 0.001$
Respiratory	23 (41.1)	11 (40.7)	$P \leq$ NS
Nervous	3 (5.4%)	8 (29.8)	$P = 0.02$
Gastrointestinal	2 (3.6)	3 (11.0)	$P =$ NS
PANCA/MPO	51 (91.1)	15 (55.5)[a]	$P < 0.001$
CANCA/PR3	0 (0.0)	9 (33.3)[a]	$P < 0.001$
Negative ANCA	5 (8.9)	2 (7.4)[a]	$P =$ NS

[a]In three cases there were no ANCA data available.
NS = not significant.

involvement, which were much less frequent in Japan (Table 1).

Discussion

This study shows for the first time major differences in the frequencies of the subtypes of renal AAV between Japan and the UK. Renal AAV in Japan is almost exclusively MPA, whereas in the UK only 41% of cases of renal AAV have MPA. The overall incidence of renal vasculitis is similar in the two populations. Our present estimate of renal vasculitis is lower than our previous figure possibly because of a tighter case definition for renal vasculitis. We looked for renal vasculitis at presentation; some patients, especially with WG, may present with renal involvement for the first time at relapse, and thus we may have underestimated the occurrence of renal vasculitis.

The aetiology of AAV is unknown, but is generally considered to be the result of an interaction between a trigger agent (as yet unknown) and a genetically predisposed host. As yet relatively little is known about the genetics of AAV in different populations because appropriate international studies using agreed methods of classification and genetic profiling have not been performed. One of the striking differences between the two populations in this study is the lack of cANCA/PR3-positive AAV in the Japanese cohort. It is possible that ANCA expression is different between populations. In Europe, WG appears to be more common in the North compared with MPA, whereas the reverse is true in Southern Europe. A recent study of 426 cases of AAV seen in a renal unit in Beijing also suggests that the ratio cANCA/PR3:pANCA/MPO positivity is 1:5 [9]. In the whole of our cohort from 2000–2004 the ratio is 2:3 and in the renal cohort 3:5. These data suggest that ANCA phenotype expression is dependent on the genetic background. It is possible therefore that the same trigger stimulus to AAV induction could result in different antigen expression in different populations. This also suggests that the tight association seen between PR3-ANCA and WG in European populations may not hold in other populations with a different genetic background.

The clinical phenotype of renal AAV also appears to be different in the two populations. ENT and neurological involvement is less common in Japan. ENT disease is a *sine qua non* of WG and its relative absence supports the serological lack of PR3 in the Japanese patients.

The major difference between the two studies is that the UK study was conducted prospectively whilst the Japanese study was retrospective and clearly there is the possibility of incomplete case capture. Furthermore, the Japanese study was performed in a renal unit whereas the UK study was performed in a rheumatology unit, and care was also taken to identify patients attending other departments. These confounders would tend to reduce the differences between the populations; however, it is unlikely that this can be solely the explanation for the serological differences seen. The EMEA algorithm was only applied in the UK, but as it employs both the ACR (1990) classification criteria and the Chapel Hill Consensus definitions, we do not, however, believe that this has confounded the results, because the differences in classification are supported by the marked difference in ANCA specificity between the two populations. It should be noted that renal-limited vasculitis is classified as MPA using the EMEA algorithm.

These data led us to hypothesize that the environmental and genetic triggers for renal vasculitis are different from those driving granulomatous disease seen predominately in the upper respiratory tract. There is little data available on the genetic backgrounds of patients with vasculitis and different clinical phenotypes. In Europe, there is a substantial overlap between ENT, renal involvement and PR3-ANCA. In Japan, in contrast, the overlap is much smaller with PR3-ANCA disease being mainly localized to the ENT tract. This could reflect a different genetic background. As yet relatively little is known about the genetics of AAV in different populations because appropriate international studies using agreed methods of classification and genetic profiling have not been performed. There is a significant association with HLA-DRB1*0901 in Japanese patients with MPA [10]; the haplotype DRB1*0901-DQB1*0303 represents the primary genetic risk for MPA within the HLA region in Japanese [11]. However, MPA has not been similarly studied in Europe. A small study (out of 16 patients only 3 had renal disease) in Japan showed an association with HLA-DR9 [12], but this has not been confirmed in Europe. In Europe HLA-DPB1*0401 is associated with an increase in granulomatous disease [13]. Equally, there could be different environmental factors.

These data clearly need to be confirmed in conventional prospective rather than retrospective first studies, but do lead us to hypothesize that there are very different drivers/triggers/aetiologies to renal vasculitis in Japan as compared with the UK and Europe (particularly northern Europe) and suggest, for example, that ENT and renal disease have quite different aetiologies in these populations. The genetic associations of both granulomatous vasculitis and renal vasculitis need to be investigated in large well-characterized cohorts.

Acknowledgement. We thank the Japanese Health Sciences Foundation for supporting this study.

Conflict of interest statement. None declared.

(See related article by P. Lamprecht and W. L. Gross. A little help from our friends: what an epidemiologic study teaches us about autoinflammation, granuloma and proteinase-3-specific antineutrophil cytoplasmic autoantibodies. *Nephrol Dial Transplant* 2008; 23: 3743–3745.)

References

1. Watts RA, Scott DGI. Epidemiology of systemic vasculitis. In: Bridges L and Ball G. (eds). *Vasculitis*, 2nd edn2nd edn. Oxford University Press, Oxford, UK: 2007; 7–21
2. Watts RA, Lane SE, Scott DG *et al.* Epidemiology of vasculitis in Europe. *Ann Rheum Dis* 2001; 60: 1156–1157
3. Hashimoto H, Yaso T, Yoshida M *et al.* Clinical and epidemiological analysis of anit-neutrophil cytoplasmic (ANCA) vasculitis from a nationwide survey in 1998 in Japan. *Annual Report of The Research Committee on Intractable Vasculitis and the Research Committee on Epidemiology on Intractable Diseases, the Ministry of Health and Welfare of Japan.* 1998, 213–229 (in Japanese)

4. Fujimoto SU, S, Hisanaga S, Fukudome K *et al.* Incidence of ANCA-associated primary real vasculitis in the Miyazaki Prefecture: the first population based, retrospective, epidemiologic survey in Japan. *Clin J Am Soc Nephrol* 2006; 1: 1016–1022
5. Lane SE, Scott DG, Heaton A *et al.* Primary renal vasculitis in Norfolk—increasing incidence or increasing recognition? *Nephrol Dial Transplant* 2000; 15: 23–27
6. Watts RA, Lane SE, Bentham G *et al.* Epidemiology of systemic vasculitis: a ten-year study in the United Kingdom. *Arthritis Rheum* 2000; 43: 414–419
7. Watts RA, Mooney J, Lane SE *et al.* Rheumatoid vasculitis: becoming extinct? *Rheumatology* 2004; 43: 920–923
8. Watts R, Lane S, Hanslik T *et al.* Development and validation of a consensus methodology for the classification of the ANCA-associated vasculitides and polyarteritis nodosa for epidemiological studies. *Ann Rheum Dis* 2007; 66: 222–227
9. Chen M, Yu F, Zhang Y *et al.* Clinical and pathological characteristics of Chinese patients with antineutrophil cytoplasmic antibody associated vasculitis: a study of 426 patients from a single centre. *Postgrad Med J* 2005; 81: 723–727
10. Tsuchiya N, Kobayashi S, Kawasaki A *et al.* Genetic background of Japanese patients with antineutrophil cytoplasmic antibody-associated vasculitis: association of HLA-DRB1*0901 with microscopic polyangiitis. *J Rheumat* 2003; 30: 1534–1540
11. Tsuchiya N, Kobayashi S, Hashimoto H *et al.* Association of HLA-DRB1*0901-DQB1*0303 haplotype with microscopic polyangiitis in Japanese. *Genes Immunity* 2006; 7: 81–84
12. Nakamaru Y, Maguchi S, Takizawa M *et al.* The association between human leukocyte antigens (HLA) and cytoplasmic-antineutrophil cytoplasmic antibody (cANCA)-positive Wegener's granulomatosis in a Japanese population. *Rhinology* 1996; 34: 163–165
13. Jagiello P, Gross WL, Epplen JT. Complex genetics of Wegener granulomatosis. *Autoimmun Rev* 2005; 4: 42–47

Received for publication: 16.11.07
Accepted in revised form: 30.5.08

Deoxynucleic Acids from *Cryptococcus neoformans* Activate Myeloid Dendritic Cells via a TLR9-Dependent Pathway[1]

Kiwamu Nakamura,[2*¶] Akiko Miyazato,[2*] Gang Xiao,[3†] Masumitsu Hatta,[*] Ken Inden,[*] Tetsuji Aoyagi,[*] Kohei Shiratori,[†] Kiyoshi Takeda,[‖] Shizuo Akira,[#] Shinobu Saijo,[**] Yoichiro Iwakura,[**] Yoshiyuki Adachi,[††] Naohito Ohno,[††] Kazuo Suzuki,[‡‡] Jiro Fujita,[¶] Mitsuo Kaku,[*‡§] and Kazuyoshi Kawakami[4†§]

The mechanism of host cell recognition of *Cryptococcus neoformans*, an opportunistic fungal pathogen in immunocompromised patients, remains poorly understood. In the present study, we asked whether the DNA of this yeast activates mouse bone marrow-derived myeloid dendritic cells (BM-DCs). BM-DCs released IL-12p40 and expressed CD40 upon stimulation with cryptococcal DNA, and the response was abolished by treatment with DNase, but not with RNase. IL-12p40 production and CD40 expression were attenuated by chloroquine, bafilomycin A, and inhibitory oligodeoxynucleotides (ODN) that suppressed the responses caused by CpG-ODN. Activation of BM-DCs by cryptococcal DNA was almost completely abrogated in TLR9 gene-disrupted (TLR9$^{-/-}$) mice and MyD88$^{-/-}$ mice, similar to that by CpG-ODN. In addition, upon stimulation with whole yeast cells of acapsular *C. neoformans*, TLR9$^{-/-}$ BM-DCs produced a lower amount of IL-12p40 than those from wild-type mice, and TLR9$^{-/-}$ mice were more susceptible to pulmonary infection with this fungal pathogen than wild-type mice, as shown by increased number of live colonies in lungs. Treatment of cryptococcal DNA with methylase resulted in reduced IL-12p40 synthesis by BM-DCs. Furthermore, using a luciferase reporter assay, cryptococcal DNA activated NF-κB in HEK293 cells transfected with the TLR9 gene. Finally, confocal microscopy showed colocalization of fluorescence-labeled cryptococcal DNA with CpG-ODN and the findings merged in part with the distribution of TLR9 in BM-DCs. Our results demonstrate that cryptococcal DNA causes activation of BM-DCs in a TLR9-dependent manner and suggest that the CpG motif-containing DNA may contribute to the development of inflammatory responses after infection with *C. neoformans*. *The Journal of Immunology*, 2008, 180: 4067–4074.

Cryptococcus neoformans (Cn)[5] is an opportunistic fungal pathogen and frequently causes fatal meningoencephalitis in patients with impaired immune responses, such as AIDS. It is an intracellular microorganism known to grow in macrophages, and has certain escape mechanisms that avoid killing by these cells (1). The host defense against Cn is largely mediated by cellular immunity (2) and CD4$^+$ T cells play a critical role in eradicating the infection (3, 4). The fate of this infection is greatly influenced by the Th1-Th2 cytokine balance: polarized Th1 immune response leads to protection, while biased synthesis of Th2 cytokines renders hosts prone to infection (5). Such balance is critically regulated by a variety of immune cells at an early stage after invasion of Cn into lung tissues, which includes dendritic cells (DCs), macrophages, NK cells, NKT cells, γδ T cells, and even neutrophils (6–10).

Invasion of microbial pathogens into tissues results in the development of inflammatory responses, which are initiated by recognition of pathogen-associated molecular patterns via pattern recognition receptors, such as TLRs (11). Using mice genetically lacking MyD88 or TLR2 (MyD88$^{-/-}$ or TLR2$^{-/-}$ mice), it was revealed that MyD88 plays a critical role while TLR2 plays a relatively limited role in the response to Cn (12). In another study (13), TLR2$^{-/-}$ mice as well as MyD88$^{-/-}$ mice succumbed to the infection, which was associated with reduced production of TNF-α, IL-2, and IFN-γ. In contrast, in our study (14), TLR2 and TLR4 did not seem to be involved in the host-protective response to this infection. In contrast, TLR4 was reported to sense glucuronoxylomannan, a major component of the polysaccharide capsule of Cn, in Chinese hamster ovary cells (15). Thus, the role of

*Department of Infection Control and Laboratory Diagnostics, Internal Medicine, Tohoku University Graduate School of Medicine, †Microbiology and Immunology, Department of Medical Technology, Tohoku University School of Health Sciences, ‡Comprehensive Research and Education Center for Planning of Drug Development and Clinical Evaluation Project, Tohoku University, and §Infection-Control Research Center, Tohoku University Hospital, Sendai; ¶Department of Medicine and Therapeutics, Control and Prevention of Infectious Diseases, Faculty of Medicine, University of the Ryukyus, Nishihara; ‖Department of Microbiology and Immunology, Graduate School of Medicine and #Department of Host Defense, Research Institute for Microbial Disease, Osaka University, Osaka; **Department of Microbiology and Immunology, Center for Experimental Medicine, Institute of Medical Science, University of Tokyo; ††Laboratory for Immunopharmacology of Microbial Products, Tokyo University of Pharmacy and Life Science, Tokyo; and ‡‡Department of Immunology, Inflammatory Program, Chiba University School of Medicine, Chiba, Japan

Received for publication July 20, 2007. Accepted for publication January 17, 2008.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by a Grant-in-Aid for Science Research (C) (18590413) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, the Ministry of Health and Welfare of Japan, and Tohoku University 21st Center of Excellence Program "Comprehensive Research and Education Center for Planning of Drug Development and Clinical Evaluation Project."

[2] K.N. and A.M. contributed equally to this work.

[3] Current address: Department of Laboratory Medicine, The 6th Affiliated Hospital, Sun Yat-sen University School of Medicine, Guangzhou, China.

[4] Address correspondence and reprint requests to Dr. Kazuyoshi Kawakami, Microbiology and Immunology, Department of Medical Technology, Tohoku University School of Health Sciences, 2-1 Seiryo-cho, Aoba-ku, Sendai, Miyagi 980-8575, Japan. E-mail address: kawakami@mail.tains.tohoku.ac.jp

[5] Abbreviations used in this paper: Cn, *Cryptococcus neoformans*; DC, dendritic cell; BM-DC, bone marrow-derived DC; ODN, oligodeoxynucleotide; WT, wild type; OX-CA, NaClO-oxidized *Candida albicans*.

Copyright © 2008 by The American Association of Immunologists, Inc. 0022-1767/08/$2.00

TLR2 and TLR4 in recognition of Cn remains to be fully understood, and some other TLR may be involved when considered along with the previous findings that the host-protective response was impaired in MyD88$^{-/-}$ mice (12, 13).

The unmethylated CpG motif-containing DNA from prokaryotic microorganisms such as bacteria and viruses is known to trigger host immune responses by interacting with TLR9 (16–18), which is distributed in the endosomal compartments (19, 20). Such interaction causes activation of signaling pathways mediated by an adaptor molecule, MyD88, leading to the synthesis of proinflammatory cytokines and expression of costimulatory molecules by macrophages and DCs (20, 21). In previous studies (22, 23), DNA from protozoa was reported to stimulate B cell proliferation and macrophage cytokine synthesis in a TLR9-dependent manner, although they are eukaryotic microorganisms. These findings raise a possibility that DNA from Cn may be involved in the activation of host immune responses, as in the case of protozoa. In agreement with this hypothesis, DNA from some fungi, such as *Schizosaccharomyces pompe* and *Paracoccidioides brasiliensis*, is also reported to stimulate a battery of immune responses (24, 25).

The main hypothesis of the present study was that DNA from Cn can induce the activation of DCs. The results showed that cryptococcal DNA stimulated bone marrow-derived DCs (BM-DCs) to synthesize cytokines and to express costimulatory molecules. These actions were completely dependent on the expression of TLR9. Furthermore, cryptococcal DNA was taken into the endosomal compartment in a manner similar to the synthetic CpG-oligodeoxynucleotide (ODN) and triggered a signaling pathway for the activation of NF-κB via TLR9.

Materials and Methods

Mice

TLR9$^{-/-}$, MyD88$^{-/-}$, Dectin-1$^{-/-}$, and TRIF$^{-/-}$ mice were generated, as described previously (18, 26–28). Homozygous mice were backcrossed to C57BL/6 mice for more than eight generations. Male or female mice at 6–10 wk of age were used for the experiments and wild-type (WT) C57BL/6 mice were used as controls. All of the mutant mice were kept under specific pathogen-free conditions at Oriental Biosciences (Kyoto, Japan), the Center for Experimental Medicine, Institute of Medical Science, University of Tokyo, and Kyusyu University, respectively. The experiments were conducted according to the institutional guidelines and were approved by the institutional ethics committees.

Microorganisms

The acapsular strain of Cn, designated as Cap67 (a gift from Dr. S. M. Levitz, Boston University, Boston, MA) and its parent strain, designated as B3501 (a gift from Dr. K. Chung, National Institutes of Health, Bethesda, MD) were used. Serotype A-encapsulated strains of Cn, designated as YC-11 and 13, were established from patients with pulmonary cryptococcosis (29). The yeast cells were cultured on potato dextrose agar plates (Eiken) for 2–3 days before use.

Pulmonary infection and enumeration of viable Cn

To induce pulmonary infection, mice were anesthetized by i.p. injection of 70 mg/kg pentobarbital (Abbott Laboratories) and restrained on a small board. Live Cn, Cap67 strain (5×10^6 cells), were inoculated at 50 μl/mouse by insertion of a 25-gauge blunt needle into and parallel to the trachea. Mice were sacrificed 2 wk after infection, and lungs and brains were dissected carefully and excised, then separately homogenized in 5 and 2 ml of distilled water, respectively, by teasing with a stainless mesh at room temperature. The homogenates, appropriately diluted with distilled water, were inoculated at 100 μl on potato dextrose agar plates, cultured for 2–3 days, followed by counting the number of colonies.

Preparation of Cn lysates and DNA

Cn yeast cells (1×10^{11}) were treated with 70% ethanol for 30 min, washed three times with PBS, and then received five cycles of disruption with 0.3 mm of zirconia beads by use of Multibeads shocker (Yasui Kikai). For preparation of Cn DNA, the yeast cells were lysed with 100 mM Tris-hydrochloride (pH 7.5), 0.5% SDS, and 30 mM EDTA at 100°C for 15 min. The DNA was purified by extraction with phenol/chloroform/isoamyl alcohol (25:24:1) and isopropanol precipitation. The pellet was washed with 70% ethanol, dried, and resolved with distilled water. The obtained DNA was kept at −20°C until use. The OD_{260}:OD_{280} ratio was usually between 1.5 and 2.0. The endotoxin content in the DNA preparations measured by *Limulus* amebocyte lysate assay was <10 pg/ml.

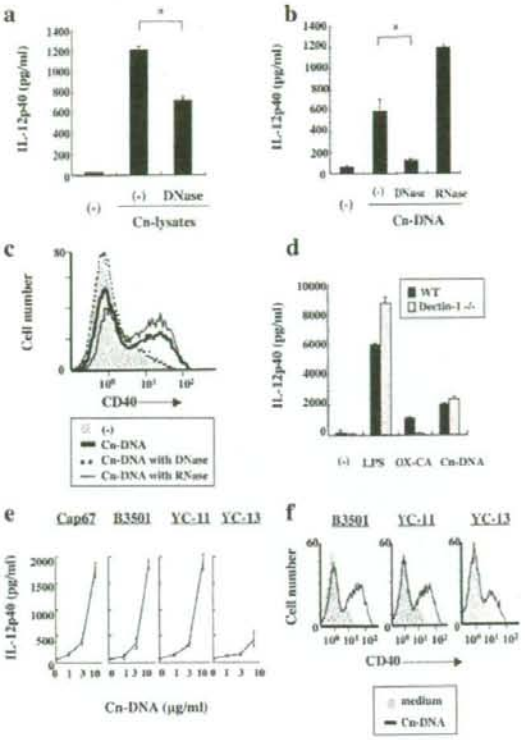

FIGURE 1. DNA from Cn activate BM-DCs. BM-DCs were cultured with various stimulants for 24 h. IL-12p40 levels were assayed by ELISA and surface expression of CD40 on BM-DCs was determined by flow cytometry. *a*, BM-DCs were cultured with 1% Cap67 lysates that were pretreated or not with DNase. *b* and *c*, BM-DCs were cultured with 10 μg/ml Cap67 DNA pretreated or not with DNase or RNase. *d*, BM-DCs from dectin-1$^{-/-}$ or WT mice were cultured with LPS (1 μg/ml), OX-CA (1 μg/ml), or Cap67 DNA (10 μg/ml). *e* and *f*, BM-DCs were cultured with DNA from Cap67, B3501, YC-11, and YC-13 at the indicated doses. Data are the mean ± SD of triplicate cultures. Flow cytometry data are representative of three independent experiments. *, $p < 0.05$.

Culture medium and reagents

RPMI 1640 medium was obtained from Nippro and FCS from Cansera. Bafilomycin A, DNase, RNase, peptidoglycan (PG), and LPS were purchased from Sigma-Aldrich, polymixin B from MP Bioscience, and chloroquine from Wako Biochemicals. NaClO-oxidized *Candida albicans* (OX-CA: a particle form β-1,6-branched β-1,3-glucan was prepared as described previously (30)). CpG-ODN (CpG1826: TCC ATG ACG TTC CTG ACG TT) and inhibitory ODN (ODN2088 and 2114). TCC TGG CGG GGA AGT and TCC TGG ACG GGA AGT, respectively, were synthesized at Hokkaido System Science. All ODN were phosphorothioated and purified by HPLC. The endotoxin content measured by *Limulus* amebocyte lysate assay was <10 pg/ml.

Preparation of Cn culture supernatants

Cn yeast cells (YC-11) were cultured at 1×10^6/ml in RPMI 1640 medium supplemented with 10% FCS, 100 U/ml penicillin G, and 100 μg/ml streptomycin for 48–72 h in a 5% CO_2 incubator. The culture supernatants were

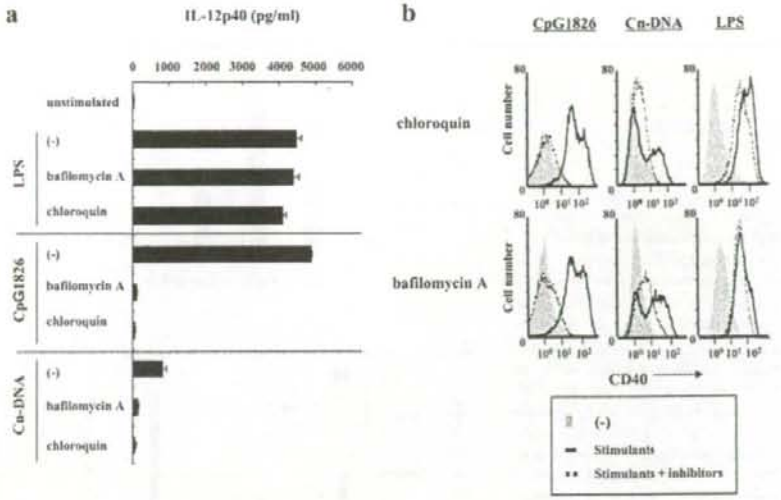

FIGURE 2. Effects of chloroquine and bafilomycin A. BM-DCs pretreated or not with chloroquine (3 μg/ml) or bafilomycin A (60 nM) were cultured with LPS (1 μg/ml), CpG1826 (150 nM), or Cap67 DNA (10 μg/ml). IL-12p40 levels in the culture supernatants (*a*) and expression of CD40 on BM-DCs (*b*) were measured. Data are mean ± SD of triplicate cultures. Flow cytometry data are representative of three independent experiments.

collected and passed through 0.2-μm Millipore filter and kept at −70°C before use.

Treatment of DNA with DNase, RNase, and methylase

Cap67 DNA was incubated with 10 μg/ml DNase or RNase in water bath at 37°C for 4 h. After incubation, the DNAs were heated at 68°C for 20 min to inactivate DNase and again purified by isopropanol precipitation. Methylated dsCpG1826 or Cap67 DNA was synthesized by incubating each DNA with Sss CpG methylase (10 U/μg DNA) for 24 h at 37°C in a water bath. Then, DNAs were heated at 68°C for 20 min to inactivate Sss CpG methylase and centrifuged by adding isopropanol for purification.

Preparation and culture of DCs

DC were prepared from BM cells as described by Lutz et al. (31), with some modifications. Briefly, BM cells from WT, TLR9KO, MyD88KO, TRIFKO, and dectin-1KO mice were cultured at 2×10^5/ml in 10 ml RPMI 1640 medium supplemented with 10% FCS (Cansera), 100 U/ml penicillin G, 100 μg/ml streptomycin, and 50 μM 2-ME containing 20 ng/ml murine GM-CSF (WakoCytomation). On day 3, 10 ml of the same medium was added, followed by a half change with the GM-CSF-containing culture medium on day 6. On day 8, nonadherent cells were collected and used as BM-DCs. The obtained cells were cultured at 1×10^5/ml with various stimulants for 24 h at 37°C in 5% CO_2 incubator.

Cytokine assay

The concentration of IL-12p40 in the culture supernatants was measured by ELISA using capture and biotinylated developing Abs (BD Biosciences). The detection limit was 15 pg/ml.

Flow cytometric analysis

Cells were preincubated with anti-FcγRII and III mAb, prepared by a protein G column kit (Kirkegaard & Perry Laboratories) from the culture supernatants of hybridoma cells (clone 2.4G2), on ice for 15 min in PBS containing 1% FCS and 0.1% sodium azide, stained with FITC-conjugated anti-CD11c mAb (clone HL3; BD Biosciences), and PE-conjugated anti-CD40 mAb (clone 1C10; eBioscience) for 25 min and then washed three times in the same buffer. Isotype-matched irrelevant Abs were used for control staining. The propidium iodide-stained population was excluded as dead cells. The stained cells were analyzed using a Cytomics FC500 flow cytometer (Beckman Coulter). Data were collected from 15,000 to 20,000 individual live cells using parameters of forward scatter/side scatter and FL1 to set a gate on the $CD11c^+$ lymphocyte population.

TLR9 cloning and NF-κB reporter assay

The full-length *tlr9* was PCR amplified from a cDNA of mouse macrophage cell line RAW264.7 cells (RIKEN Cell Bank). Specific primers for amplification of *tlr9* were designed based on the GenBank accession number AF314224 (32). The sequences of primers were 5′-CAC CAT GGT TCT CCG TCG AAG G-3′ (forward) and 5′-CTA TTC TGC TGT AGG TCC CCG GC-3′ (reverse). The amplified full-length cDNA was cloned

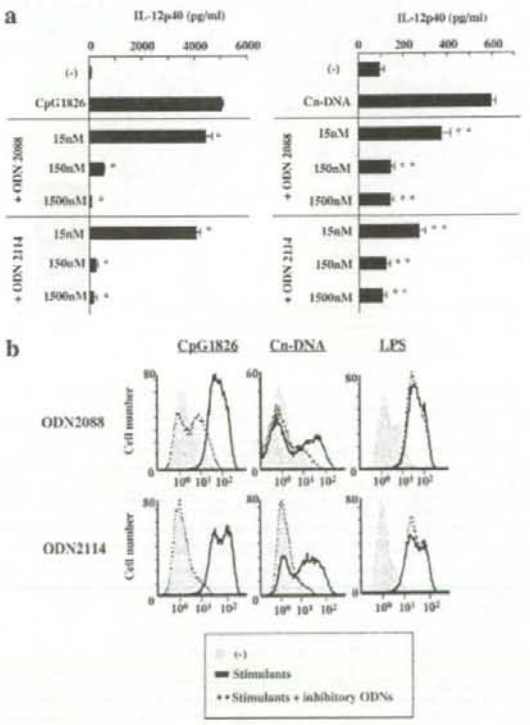

FIGURE 3. Effect of inhibitory ODN. BM-DCs were cultured with CpG1826 (150 nM) or Cap67 DNA (10 μg/ml) in the presence or absence of the indicated doses of ODN2088 or ODN2114. IL-12p40 levels in the culture supernatants (*a*) and expression of CD40 on BM-DCs (*b*) were measured. Data are mean ± SD of triplicate cultures. * and **, $p < 0.05$, compared with CpG1826 and Cn-DNA, respectively. Flow cytometry data are representative of three independent experiments.

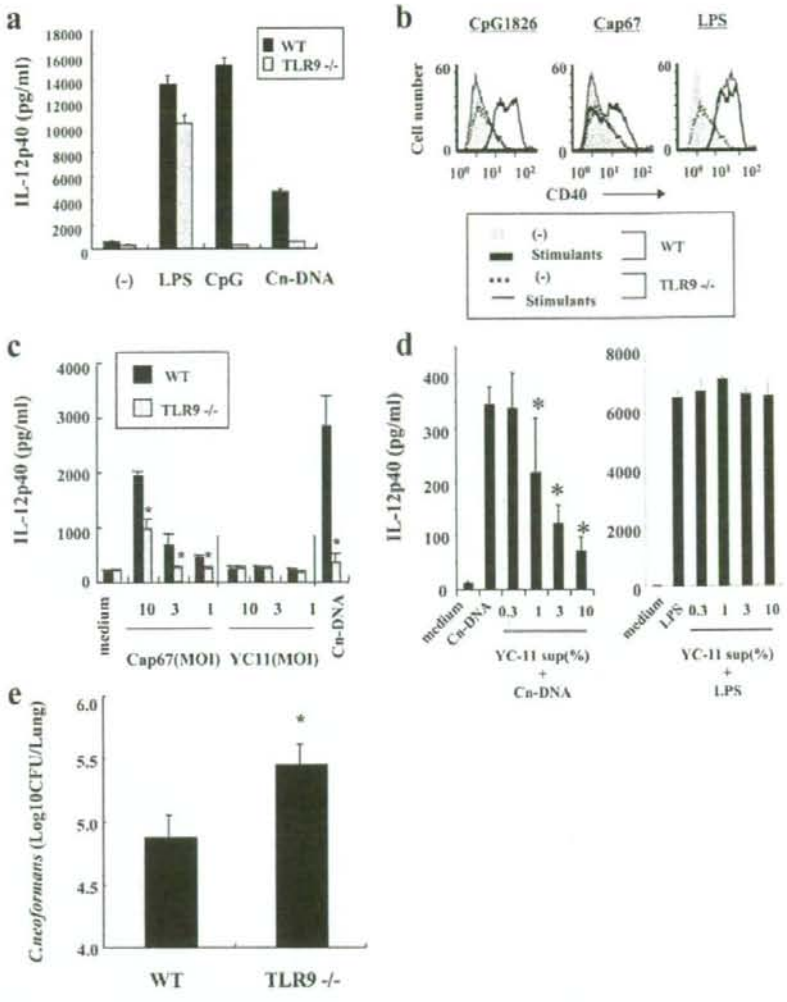

FIGURE 4. Cryptococcal DNA require TLR9 for activation of BM-DCs. BM-DCs from TLR9$^{-/-}$ or WT mice were cultured with LPS (1 μg/ml), CpG1826 (150 nM), or Cap67 DNA (10 μg/ml). IL-12p40 levels in the culture supernatants (*a*) and expression of CD40 on BM-DCs (*b*) were measured. Data are mean ± SD of triplicate cultures. Flow cytometry data are representative of two independent experiments. *c*, BM-DCs from TLR9$^{-/-}$ or WT mice were cultured with the indicated doses of Cap67 or YC-11 for 24 h, in which the extracellular yeast cells were not washed away. IL-12p40 levels in the culture supernatants were measured. Data are mean ± SD of triplicate cultures. MOI, Multiplicity of infection. *, $p < 0.05$, compared with WT mice. *d*, BM-DCs from WT mice were cultured with Cap67 DNA (10 μg/ml) or LPS (1 μg/ml) in the presence or absence of the indicated doses of YC-11 culture supernatants for 24 h, and the concentrations of IL-12p40 in the culture supernatants were measured. Data are mean ± SD of triplicate cultures. sup, Culture supernatants. *, $p < 0.05$, compared with the cultures without YC-11 culture supernatants. *e*, TLR9$^{-/-}$ or WT mice were infected intratracheally with Cap67 (5×10^6/mouse) and the number of live colonies in lungs was counted on day 14 after infection. Data are mean ± SD of seven mice. *, $p < 0.05$, compared with WT mice.

into pcDNA6.2/TOPO (Invitrogen Life Technologies) and sequenced. Endothelial leukocyte adhesion molecule 1 luciferase reporter plasmids were provided by Dr. M. Mitsuyama (Kyoto University, Kyoto, Japan). For the NF-κB luciferase reporter assay, HEK293T cells were transiently transfected with 100 ng of reporter plasmid along with 100 ng of *tlr9* expression plasmids or empty control plasmid. At 24 h after transfection, the cells were treated with PG, CpG-ODN, or cryptococcal DNA for 6 h. Luciferase activity in the total cell lysate was measured with the dual-luciferase reporter assay system (Promega). The *Renilla*-luciferase reporter gene was simultaneously transfected as an internal control. Relative luciferase activities were calculated as folds of induction compared with unstimulated vector control.

Florescent labeling and confocal imaging Cap67

DNA was labeled with Alexa Fluor 647 using ULYSIS Nucleic Acid Labeling kits (Molecular Probes) according to the instructions provided by the manufacturer. The relative efficiency of a labeling reaction was evaluated by calculating the approximate ratio of bases to dye molecules. Rhodamine-labeled CpG-ODN was purchased from Hokkaido System Science. FITC-conjugated TLR9 mAb (clone 26C593.2) was purchased from IMGENEX. Cells were incubated in microtubes, cytospun to glass slides, and coverslips were mounted on the glass slides with a ProLong Antifade Kit (Molecular Probes). Confocal studies were performed with an oil immersion objective (×60 Plan Apo; numerical aperture 1.4) and a Nikon TIRF-C1 confocal microscope. The software, Nikon EZ-C1 version 2.00, were used to acquire and process the confocal images. Dual-color images were acquired using a sequential acquisition mode to avoid cross-excitation.

Statistical analysis

Analysis was conducted using Statview II software (Abacus Concept) on a Macintosh computer. Data are expressed as mean ± SD. Differences between groups were examined for statistical significance using one-way ANOVA with a post hoc analysis (Fisher (plausible least significant difference) PLSD test). A value of $p < 0.05$ was considered significant.

Results

DNA from Cn activates BM-DCs

We measured the activity of Cn to induce the synthesis of IL-12p40 by BM-DCs. For this purpose, we used the acapsular mutant strain Cap67 to avoid the influence of capsular polysaccharides, which were reported to suppress various immune responses (33). The lysates of Cap67 prepared using a multibeads shocker generated IL-12p40 by BM-DCs. We tested the effect of DNase on the cytokine-inducing capability of Cap67 lysates. As shown in Fig. 1*a*, DNase significantly reduced the production of both cytokines.

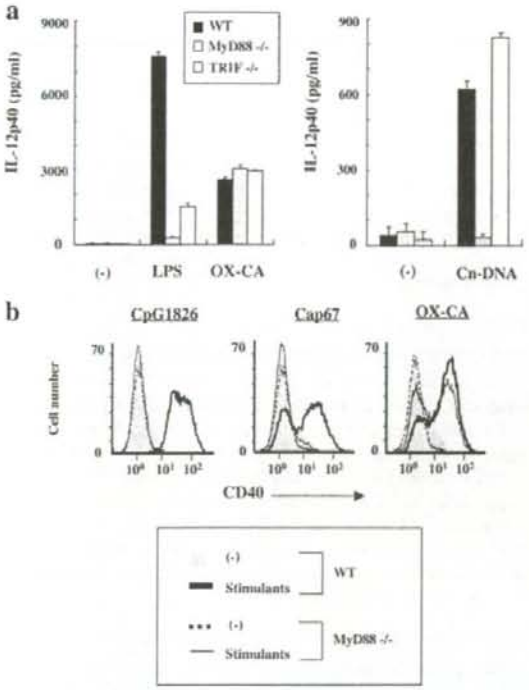

FIGURE 5. Cryptococcal DNA requires MyD88 for activation of BM-DCs. BM-DCs from MyD88$^{-/-}$, TRIF$^{-/-}$, or WT mice were cultured with LPS (1 μg/ml), OX-CA (1 μg/ml), or Cap67 DNA (10 μg/ml). *a*, IL-12p40 levels in the culture supernatants were measured. Data are mean ± SD of triplicate cultures. *b*, Flow cytometry data are representative of two independent experiments.

These results suggest the involvement of Cap67 DNA in this response. Next, we tested whether Cap67-derived DNA can activate BM-DCs by measuring the synthesis of IL-12p40 and expression of CD40 on these cells. IL-12p40 was produced by this stimulation in a dose-dependent manner and such production was completely abolished by the addition of DNase, but not of RNase (Fig. 1*b*). Similarly, the expression of CD40 on BM-DCs was accelerated by coculture with Cap67 DNA, and this effect was inhibited by DNase, but not by RNase (Fig. 1*c*). These responses were not affected by the addition of polymixin B, which completely abolished cytokine production induced by LPS (data not shown), indicating that the activity of Cap67 DNA was not mediated by LPS contamination. Since fungi secrete β-glucan that activates BM-DCs (34), it is possible that β-glucan contaminating the DNA preparation could have mediated these responses. However, this was not the case, because IL-12p40 produced by Cap67 DNA-stimulated BM-DCs was not decreased in mice lacking dectin-1, the sole receptor of β-glucan (Fig. 1*d*). Furthermore, we tested also whether these findings were specific for this particular mutant strain of Cn. IL-12p40 synthesis by BM-DCs was induced by DNA derived from the parent strain of Cap67, B3501, and two clinically isolated strains, YC-13 and YC-11, in a dose-dependent fashion (Fig. 1*e*). Similarly, the expression of CD40 on BM-DCs was enhanced by DNA from these strains (Fig. 1*f*). These results indicate that DNA from Cn directly activated BM-DCs to synthesize the cytokine and to express the surface activation Ag. In contrast, DNA derived from mouse splenocytes and human PBMC did not induce these responses by BM-DCs (data not shown).

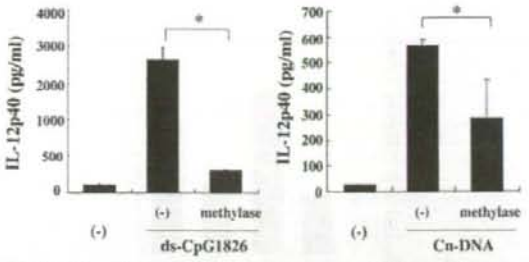

FIGURE 6. Effect of methylation of cryptococcal DNA. BM-DCs were cultured with dsCpG1826 (4.5 μM) or Cap67 DNA (10 μg/ml) pretreated or not with methylase. IL-12p40 levels in the culture supernatants were measured. Data are mean ± SD of triplicate cultures. *, $p < 0.05$.

DNA of Cn activates BM-DCs in a manner similar to CpG-ODN

Because Cn is a eukaryote, we questioned whether the activity of DNA from this fungal pathogen is different from that of a synthetic ODN containing the CpG motif, which prokaryote DNA usually carries in an unmethylated form (17). Previous studies reported that CpG-ODN-induced activation of BM-DCs was strongly inhibited by chloroquine and bafilomycin A, both of which interfere with endosomal maturation (35). Therefore, to address this issue, we compared the effects of these inhibitors on the synthesis of IL-12p40 by BM-DCs and expression of CD40 caused by a stimulatory CpG-ODN (CpG1826) and Cap67 DNA. As shown in Fig. 2*a*, the synthesis of IL-12p40 by Cap67 DNA-stimulated cells was strongly suppressed by addition of either chloroquine or bafilomycin A; such an effect was similar to the responses induced by CpG1826, but not by LPS. Similarly, the addition of chloroquine or bafilomycin A suppressed CD40 expression induced by Cap67 DNA and CpG1826, but not by LPS (Fig. 2*b*).

Previous studies identified DNA sequences that neutralized the CpG-ODN-induced activation of B cells (36). In the next experiments, therefore, we tested the effect of such ODN with an inhibitory DNA motif on the induction of IL-12p40 synthesis and CD40 expression by BM-DCs. As shown in Fig. 3, ODN2088 and ODN2114 strongly inhibited these responses induced by both CpG1826 and Cap67 DNA, whereas such inhibition was not detected on the responses by LPS. These results indicate that Cn DNA exerted its activity in a manner similar to CpG1826 and suggest that the fungal DNA may activate BM-DCs via interaction with TLR9, a recognition receptor of the unmethylated CpG motif (17, 18).

Cn DNA requires TLR9 for activation of BM-DCs

To address this possibility, we investigated the role of TLR9 in Cap67 DNA-induced BM-DC activation. As shown in Fig. 4, *a* and *b*, production of IL-12p40 and expression of CD40 by Cap67 DNA- and CpG1826-stimulated cells were completely diminished in TLR9$^{-/-}$ mice, whereas such effect was not detected when the cells were stimulated with LPS. In this study, it should be noted that DNA was not released from Cn outside the DCs, but rather likely generated from the killed yeast cells in the endosomal compartments. Therefore, our current observations indicating the activation of BM-DCs by cryptococcal DNA have a more physiological significance, when defective expression of TLR9 interferes with activation induced by whole Cn. As shown in Fig. 4*c*, the amount of IL-12p40 synthesized by BM-DCs in response to whole yeast cells of Cap67, an acapsular strain, was significantly reduced in TLR9$^{-/-}$ mice compared with WT control mice, whereas YC-11, a highly encapsulated strain, failed to induce the production of