その他の検査において認められた所見

検査名　　□所見なし　□有（結果　　）

検査名　　□所見なし　□有（結果　　）

検査名　　□所見なし　□有（結果　　）

臨床経過の概要（臨床経過表を添付して頂ければ助かります。個人を特定できる箇所は消去願います）

CGD 腸炎に対する治療

□ペンタサ　（開始年齢　　歳　　か月）　使用量＿＿＿＿＿mg／kg

□　緩解　□改善　□無効

特に改善した症状　（　　）

□サラゾピリン　（開始年齢　　歳　　か月）　使用量＿＿＿＿＿mg／kg

□　緩解　□改善　□無効

特に改善した症状　（　　）

□ステロイド　（開始年齢　　歳　　か月）　使用量＿＿＿＿mg／kg

□　緩解　□改善　□無効

特に改善した症状　（　　）

□免疫抑制薬（　　）（開始年齢　　歳　　か月）使用量＿＿＿＿mg／kg

□　緩解　□改善　□無効

特に改善した症状　（　　）

□プロバイオティク等（　　）（開始年齢　　歳　　か月）　使用量＿＿＿＿mg／kg

□　緩解　□改善　□無効

特に改善した症状　（　　）

□その他（薬剤名　　）（開始年齢　　歳　　か月）　使用量＿＿＿＿mg／kg

□　緩解　□改善　□無効

特に改善した症状　（　　）

血液検査結果（腸炎発症時と重症化した時期）		
腸炎発症時、重症化した時期	歳　　ヶ月、	歳　　ヶ月
赤血球		
Hb		
血小板		
白血球数		
好中球	%	%
好酸球	%	%
リンパ球	%	%
単球	%	%
リンパ球分画		
ＣＤ３陽性細胞数	%	%
ＣＤ１９陽性細胞数	%	%
ＣＤ１６／ＣＤ５６陽性細胞数	%	%
ＣＤ４／8 比		
CRP		
ESR		

その他 CGD 腸炎に対する診断・治療についてご意見があればご記入下さい。

アンケートは以上です。ありがとうございました。

Ⅴ．班会議・第18回食細胞機能異常症研究会プログラム

厚生労働科学研究費補助金　難治性疾患克服研究事業
「外胚葉形成不全免疫不全症の実態調査と治療ガイドラインの作成」班

平成22年度班会議プログラム

日　時：平成22年12月11日（土）10時より
場　所：東京慈恵会医科大学 高木会館5階 D-1 会議室

議題・報告

1. 開催の挨拶・研究事業の進捗状況と次年度計画
　　研究代表者　国立成育医療研究センター研究所成育遺伝研究部　　小野寺雅史

2. 各班員の報告

(1) 難治性腸炎に対する抗TNFα モノクローナル抗体による治療
　　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　　布井　博幸

(2) 慢性肉芽腫症（CGD）の炎症性腸炎を含めた肉芽腫の治療に関して
　　北海道大学大学院医学研究科・小児科学　　有賀　　正

(3) 難治性消化管肉芽腫症を合併した慢性肉芽腫症に対する骨髄移植
　　広島大学大学院医歯薬学総合研究科・小児科学　　小林　正夫

(4) 国立成育医療研究センターにおける炎症性腸炎のまとめ
　　国立成育医療研究センター研究所成育遺伝研究部　　河合　利尚

(5) 小児腸管 Behcet 病5例の臨床像と治療経過
　　埼玉県立小児医療センター総合診療科　　鍵本　聖一

(6) NEMO 異常症における reversion mosaicism の検討
　　京都大学大学院医学研究科・小児科学　　西小森隆太

(7) ガレクチン9による炎症制御機構の解析
　　香川大学医学部・免疫病理学　　平島　光臣

(8) CGD の肉芽腫形成におけるオステオポンチンの関与
　　関西医科大学小児科　　蓮井　正史

(9) NEMO 異常症についてのアンケート調査研究
　　国立成育医療研究センター研究所臨床研究センター　　瀧本　哲也

3. 事務連絡

第18回食細胞機能異常症研究会

日時:　平成22年12月11日(土)13:00より
場所:　東京慈恵会医科大学　大学1号館　5階講堂

13:00-13:10

開会の挨拶　　食細胞機能異常症研究会　会長　布井博幸 先生

13:10 ー14:00

I. 機能解析　　座長　北海道大学　有賀正 先生

(1)小児疾患における骨免疫学の応用

(広島市民病院/安井耕三)

(2)好中球の細胞走化性と活性酸素産生の同時測定法の開発:
TAXIScan技術の臨床応用を目指して

((株)ECI/土屋朋子)

(3) TAXIScan-FLを用いた慢性肉芽腫症好中球機能解析

(成育/河合利尚)

14:00 ー14:20

II. 食細胞機能異常症レビュー　　座長　成育医療センター　河合利尚 先生

(4)先天性好中球減少症の最近の話題:
WHIM症候群とG6PC3欠損症

(関西医大/谷内昇一郎)

14:20 ー14:55

III. 炎症性腸疾患　　座長　成育医療センター　小野寺雅史 先生

(5)治療に難渋した慢性肉芽腫症腸炎の1例

(関西医大/居原田安奈)

(6)慢性肉芽腫症における非感染性腸炎の合併

(成育/村山静子)

～ 休憩（20分間）～

15:15 －16:20

IV.慢性肉芽腫症　　座長　関西医科大学　蓮井正史 先生

（7）乳児期に難治性Aspergillus肺炎で死亡したX連鎖性慢性肉芽腫症の一例
（北海道大/山崎康博）

（8）長期間抗菌薬投与によって治癒しえた肝膿瘍合併慢性肉芽腫症の一例
（富山大 /西田直徳）

（9）繰り返す頚部リンパ節炎を契機に慢性肉芽腫症と診断された1女児例
（愛知医大 /石澤恵）

（10）慢性肉芽腫症症例に対する播種性BCG感染症を中心とした
抗酸菌感染症治療指針作成の提案
（九州大/保科隆之）

16:20 －17:00

V. 特別講演　　座長　関西医科大学　谷内 昇一郎 先生

『先天性好中球減少症：最近の知見』

広島大学大学院医歯薬学総合研究科
小児科学研究室
教授　小林正夫 先生

17:00-17:10

閉会の挨拶　　成育医療センター　小野寺 雅史 先生

＊研究会終了後、意見交換会を予定しております。

VI. 研究成果の刊行に関する一覧表

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
Tozuka Y, Kumon M, Wada E, Onodera M, Mochizuki H, Wada K.	Material obesity impairs hippocampal BDNF production and spatial learning performance in young mouse offspring.	Neurochem Int	57(3)	235-247	2010
Hirata Y Hamanaka S, Onodera M.	Transactivation of the dopamine receptor 3 gene by a single provirus integration results in development of B cell lymphoma in transgenic mice generated from retrovirally transduced embryonic stem cells.	Blood	115 (19)	3930-3938	2010
Miyamoto N, Tanaka R, Shimura H, Watanabe T, Mori H, Onodera M, Mochizuki H, Hattori N, Urabe T.	Phosphodiesterase III inhibition promotes differentiation and survival of oligodendrocyte progenitors and enhances regeneration of ischemic white matter lesions in the adult mammalian brain.	J Cereb Blood FlowMetab	30 (2)	299-310	2010
Shirao K, Okada S, Tajima G, Tsumura M, Hara K, Yasunaga S, Ohtsubo M, Hata I, Sakura N, Shigematsu Y, Takihara Y, Kobayashi M.	Molecular pathogenesis of a novel mutation, G108D, in short-chain acyl-CoA dehydrogenase identified in subjects with short-chain acyl-CoA dehydrogenase deficiency.	Hum Genet.	127 (6)	619-628	2010
Kihara H, Ohno N, Karakawa W, Mizoguchi Y, Fukuhara R, Hayashidani M, Nomura S, Nakamura K, Kobayashi M.	Significance of immature platelet fraction and CD41-positive cells at birth in early onset neonatal thrombocytopenia.	Int J Hematol.	91(2)	245-251	2010
溝口洋子, 岡田 賢, 小林正夫	血液疾患における病態解析研究の進歩：先天性好中球減少症発症機構解明の進展	血液・腫瘍科	60(2)	118-124	2010
溝口洋子, 小林正夫	好中球減少症	小児科	51	958-994	2010
Yamada M, Arai T, Oishi T, Hatano N, Kobayashi I, Kubota M, Suzuki N, Yoda M, Kawamura N, Ariga T.	Determination of the deletion breakpoints in two patients with contiguous gene syndro me encompassing CYBB gene.	Eur J Med Genet,	53	383-388	2010

Roos D, Kuhns DB, Maddalena A, Roesler J, Lopez JA, Ariga T, Avcin T, de Boer M, Bustamante J, Condino-Neto A, Di Matteo G, He J, Hill HR, Holland SM, Kannengiesser C, Köker MY, Kondratenko I, van Leeuwen K, Malech HL, Marodi L, Nunoi H, Stasia MJ, Ventura AM, Witwer CT, Wolach B, Gallin J.	Hematologically important mutations: X-linked chronic granulomatous disease (third update).	Blood Cells Mol Dis.	45(3)	246-265	2010
Kato I, Umeda K, Awaya T, Yui Y, Niwa A, Fujino H, Matsubara H, Watanabe K, Heike T, Adachi N, Endo F, Mizukami T, Nunoi H, Nakahata T, Adachi S.	Successful treatment of refractory donor lymphocyte infusion-induced immune-mediated pancytopenia with rituximab.	Pediatr Blood Cancer.	54(2)	329-331	2010
Moritake H, Shimonodan H, Marutsuka K, Kamimura S, Kojima H, Nunoi H.	C-MYC rearrangement may induce an aggressive phenotype in anaplastic lymphoma kinase positive anaplastic large cell lymphoma: Identification of a novel fusion gene ALO17/C-MYC.	Am J Hematol.	86(1)	75-78	2011
Sakai H, Ito S, Nishikomori R, Takaoka Y, Kawai T, Saito M, Okafuji I, Yasumi T, Heike T, Nakahata T.	A case of early-onset sarcoidosis with a six-base deletion in the NOD2 gene.	Rheumatology	49(1)	194-196	2010
Kambe N, Satoh T, Tanizaki H, Fujisawa A, Saito MK, Nisikomori R.	Enhanced NF-kappaB activation with an inflammasome activator correlates with activity of autoinflammatory disease associated with NLRP3 mutations outside of exon 3: comment on the article by Jeru et al.	Arthritis Rheum	62 (10)	3123-3124	2010
Kambe N, Nakamura Y, Saito M, Nishikomori R.	The inflammasome, an innate immunity guardian, participates in skin urticarial reactions and contact hypersensitivity.	Allergol Int	59	105-113	2010
Kaneko K, Tanaka S, Hasui M, Nozu K, Krol RP, Iijima K, Sugimoto K, Takemura T.	A family with X-linked benign familial hematuria.	Pediatr Nephrol	25(3)	545-548	2010

Kaneko K, Hasui M, Hata A, Hata D, Nozu K,	Focal segmental glomerulosclerosis in a boy with Dent-2 disease.	Pediatr Nephrol	25(4)	781-782	2010
蓮井正史、金子一成	輸液療法	小児内科	42	265-270	2010
Arikawa T, Saita N, Oomizu S, Ueno M, Matsukawa A, Katoh S, Kojima K, Nagahara K, Miyake M, Yamauchi A, Kohrogi H, and Hirashima M.	Galectin-9 expands immunosuppressive macrophages to ameliorate T cell-mediated lung inflammation.	Eur J Immunol	40(2)	548-558	2010
Kobayashi T, Kuroda J, Ashihara E, Oomizu S, Terui Y, Taniyama A, Adachi S, Takagi T, Yamamoto M, Sasaki N, Horiike S, Hatake K, Yamauchi A, Hirashima M, Taniwaki M	Galectin-9 exhibits anti-myeloma activity through JNK and p38 MAP kinase pathways.	Leukemia	24 (4)	843-850	2010
Tanikawa R, Tanikawa T, Hirashima M, Yamauchi A, Tanaka Y.	Galectin-9 induces osteoblast differentiaion through the CD44/Smad signaling pathway.	BBRC	394 (2)	317-322	2010
Katoh S, Nobumoto A, Matsumoto N, Matsumoto K, Ehara N, Niki T, Inada H, Nishi N, Yamauchi A, Fukushima K, Hirashima M.	Involvement of galectin-9 in lung eosinophilia in patients with eosinophilic pneumonia.	Int Arch Allergy Immunol.	153 (3)	294-302	2010
Kuroda J, Yamamoto M, Nagoshi H, Kobayashi T, Sasaki N, Shimura Y, Horiike S, Kimura S, Yamauchi A, Hirashima M, Taniwaki M.	Targeting activating transcription factor 3 by galectin-9 induces apoptosis and overcomes various types of treatment resistance in chronic myelogenous leukemia.	Mol Cancer Res.	8(7)	994-1001	2010
Kadowaki T, Inagawa H, Kohchi C, Hirashima M, Soma G.	Preparation of lipopolysaccharide derived from Pantoea agglomerans labeled with fluorescence as a tracer for kinetics analysis.	Anticancer Res	30(8)	3151-3157	2010
Dardalhon V, Anderson AC, Karman J, Apetoh L, Chandwaskar R, Lee DH, Cornejo M, Nishi N, Yamauchi A, Quintana FJ, Sobel RA, Hirashima M, Kuchroo VK.	Tim-3/galectin-9 pathway: regulation of Th1 immunity through promotion of CD11b+Ly-6G+ myeloid cells.	J. Immunol.	185	1383-1392	2010

Mishra R, Grzybek M, Niki T, Hirashima M, Simons K.	Galectin-9 trafficking regulates apical-basal polarity in Madin-Darby canine kidney epithelial cells.	PNAS	107 (41)	17633-17638	2010
Mengshol JA, Golden-Mason L, Arikawa T, Smith M, Niki T, McWilliams R, Randall JA, McMahan R, Zimmerman MA, Rangacharii M, Dobrinskikh E, Busson P, Polyak SJ, Hirashima M, Rosen HR.	A crucial role for Kupffer cell-derived galectin-9 in regulation of T cell immunity in chronic hepatitis C infection.	Plos One	5(3)	e9504	2010
Sehrawat S, Reddy PB, Rajasagi N, Suryawanshi A, Hirashima M, Rouse BT.	Galectin-9/TIM-3 interaction regulates virus-specific primary and memory CD8+ T cell response.	PLOS Pathogens	6(5)	e1000882	2010
Horlacher T, Oberli MA, Werz DB, Kröck L, Bufali S, Mishra R, Sobek J, Simons K, Hirashima M, Niki T, Seeberger PH.	Determination of carbohydrate-binding preferences of human galectins with carbohydrate microarrays.	ChemBioChem	11 (11)	1563-1573	2010
Chagan-Yasutan H, Shiratori B, Siddiqi UR, Saitoh H, Ashino Y, Arikawa T, Hirashima M, Hattori T.	The increase of plasma galectin-9 in a patient with insulin allergy: a case report.	Clin Mol Allergy	8	12(1-4)	2010
Yoshida H, Teraoka M, Nishi N, Nakakita S, Nakamura T, Hirashima M, Kamitori S.	X-ray structures of human galectin-9 C-terminal domain in complexes with a biantennary oligosaccharide and sialyllactose.	J Biol Chem	285 (47)	36969-36976	2010
Sakai K, Kawata E, Ashihara E, Nakagawa Y, Yamauchi A, Yao H, Nagao R, Tanaka R, Yokota A, Takeuchi M, Hirai H, Kimura S, Hirashima M, Yoshimura N, Maekawa T.	Galectin-9 ameliorates acute GVH disease through the induction of T-cell apoptosis.	Eur J Immunol	41(1)	67-75	2011
Vega-Carrascal I, Reeves EP, Niki T, Arikawa T, McNally P, O'Neill SJ, Hirashima M, McElvaney NG.	Dysregulation of TIM-3/Galectin-9 pathway in the cystic fibrosis airways.	J. Immunol	186 (5)	2897-2909	2011

Iqbal AJ, Sampaio A L, Maione F, Greco K V, Niki T, Hirashima M, Perretti M, Cooper D.	Endogenous galectin-1 in a murine model of paw edema: emerging notion of galectin-9 pro-resolving effect.	Am J Pathol.	178 (3)	1201-1209	2011
高野忠将, 岩間達, 関島俊雄, 鍵本聖一	一過性偽性低アルドステロン症の3例	日本小児救急医学会雑誌	9	54-57	2010
田辺行敏, 小西健一郎, 西田裕哉, 藤澤ますみ, 谷口博子, 川畑建, 宮林寛, 菅野啓一, 清水正樹, 鍵本聖一, 城宏輔, 竹下和秀, 西本創	新生児期にエコーウイルス6型の垂直感染により重症化を認めた新生児例	埼玉県医学会雑誌	44	513-519	2010
余田篤, 友政剛, 小林昭夫, 虻川大樹, 牛島高介, 鍵本聖一, 今野武津子, 清水俊明, 田尻仁, 永田智, 藤澤卓爾, 内田恵一, 根津理一郎, 井上詠, 杉田昭, 鈴木康夫, 上野文昭, 日本IBD研究会.	炎症性腸疾患(IBD) 治療の進歩とQOL 潰瘍性大腸炎の内科治療 小児のエビデンスとコンセンサスを統合した潰瘍性大腸炎の診察ガイドラインを中心に	日本小児栄養消化器肝臓学会雑誌	23	102-106	2010
金井宏明 西崎直人, 平野大志, 藤永周一郎, 鍵本聖一	高度の体重増加不良、低蛋白血症、電解質異常をきたした重症アトピー性皮膚炎の1例	日本小児腎不全学会雑誌	30	192-194	2010
大場大樹, 岩間達, 関島俊雄, 鍵本聖一, 浜野晋一郎	薬剤アレルギーを併発し診断、治療に難渋した脳梁膨大部病変を伴う急性脳症の女児例	小児科臨床	63	2367-2371	2010
岩間達, 鍵本聖一	ランソプラゾールの内服により高ガストリン血症をきたした2幼児例	日本小児栄養消化器肝臓学会雑誌	24	27-31	2010
Iwama I, Kagimoto S, Takano T, Sekijima T, Kishimoto H, Oba A.	Case of pediatric Ménétrier disease with cytomegalovirus and Helicobacter pylori co-infection.	Pediatr Int	52	e200-203	2010
鍵本聖一	肝性脳症、肝不全	小児科診療	89	253-259	2011

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
有賀　正	全身に見られる症候　易感染症	山口徹、北原光夫、福井次矢	今日の治療指針	医学書院	東京	2011	1196-1197
有賀　正	全身に見られる症候　易感染症	金澤一郎、永井良三	今日の診断指針第六版	医学書院	東京	2010	67-70
鍵本聖一	蛋白漏出性胃腸症	児玉浩子、玉井浩、清水俊明	小児臨床栄養学	診断と治療社	東京	2010	209
鍵本聖一	経腸栄養	児玉浩子、玉井浩、清水俊明	小児臨床栄養学	診断と治療社	東京	2010	374
鍵本聖一	おもな栄養輸液製剤と経腸栄養剤	児玉浩子、玉井浩、清水俊明	小児臨床栄養学	診断と治療社	東京	2010	459

学会発表

発表者氏名	発表タイトル名	学会名	場所	年月日
小野寺雅史	遺伝子治療における我が国と欧米の違い	第1回国際協力遺伝病遺伝子治療フォーラム（特別講演）	東京	2011.1.26
Hirashima M.	Galectin-9 beneficially modulates macrophage functions in inflammation and cancer.	18th International Symposium on Molecular Cell Biology of Macrophages	Kumamoto	2010.5.20
平島光臣	ガレクチン9とマクロファージ.	第9回四国免疫フォーラム	愛媛 東温市	2010.6.19
Oomizu S, Arikawa T, Niki T, Yamauchi A, Hirashima M.	Galectin-9 down-regulates Th17 cell differentiation independently of Tim-3/Galectin-9 interaction.	第14回国際免疫学会議	神戸市	2010.8.23
平島光臣.	Galectin-9とTim-3による免疫制御機構について	東京医科歯科大学大学院	東京	2010.10.26
Hirashima M.	Galectin-9 in autoimmune pancreatitis and pancreatic cancer.	18th Yonsei International Gastroenterology Symposium.	Seoul, Korea	2010.12.4

Ohno N, Kajiume T, Hayakawa S, Kobayashi Y, Ytsunomiya A, Kobayashi M	Umbilical cord blood-derived stem cells cultured under low oxygen tension enhance the migratioin and homing efficacy through the increased expression of CXCR4.	American Society of Hematology, 52nd Annual Meeting,	Orlando, Florida, USA	2010.12.5
Karakawa S, Okada, S, Tsumura M, Yasunaga S, Ohtsubo M, Kawai T, Nishikomori R, Takihara Y, Kobayashi M	Decreased expression in NF-kB essential modulator due to a novel splice-site mutation causes ectodermal dysplasia with immunodeficiency.	American Society of Hematology, 52nd Annual Meeting,	Orlando, Florida, USA	2010.12.5
Tsumura M, Okada S, Mizoguchi Y, Sakai H, Nishikomori R, Yasunaga S, Ohtsubo M, Murata T, Obata H, Yasumi T, Heike T, Nakahata T, Takihara Y, Casanova J, Kobayashi M	A novel mutation K673R in STAT1 impaired the STAT1 signal transduction in a dominat-negative manner identified in a Japanese boy with MSMD.	14th International Congress of Immunogy, Kansai	Kobe	2010.8.22
有賀　正	日常診療における原発性免疫不全症	千歳市小児科医会講演会（特別講演）	千歳	2010.10.15
有賀　正	原発性免疫不全症に対する遺伝子治療の現状と問題点	第17回大分小児アレルギー研究会(特別講演)	大分	2010.10.22
河合 朋樹，西小森 隆太，阿部純也，横山 宏司，井澤 和司，田中尚子，酒井 秀政，村田 祐樹，八角高祐，平家 俊男.	外肺葉形成不全免疫不全症に高頻度にみられるリバージョンモザイキズムの検討.	第 113 回日本小児科学会	盛岡	2010.4.24
蓮井正史，磯崎夕佳，木全貴久，金子一成.	ニューモシスチス肺炎で致死的経過を辿った IgA 腎症の 1 例.	第 42 回日本小児感染症学会	仙台	2010.11.27
馬場洋介、関野将行，長田浩平，岩間達，関島俊雄，鍵本聖一，岸本宏志	蛋白漏出性胃腸症の内視鏡像の検討	第 113 回日本小児科学会学術集会	盛岡	2010.4.23

関野将行、馬場洋介、長田浩平、岩間達、関島俊雄、鍵本聖一	臨床像と病理組織所見に乖離がみられた牛乳アレルギーの新生児例	第113回日本小児科学会学術集会	盛岡	2010.4.23
鍵本聖一、関島俊雄、岩間達、馬場洋介、長田浩平、関野将行	臓器不全におけるミトコンドリア障害の診断と治療	第24回日本小児救急医学会	京都	2010.5.28
岩間達、鳥羽山寿子、鍵本聖一	当初クローン病と診断し その 2 年後にベーチェット病の診断に至った 1 例	IBD Club Jr	東京	2010.5.28
岩間達、鳥羽山寿子、鍵本聖一	小児大腸内視鏡検査の前処置についての検討	第37回小児内視鏡研究会	東京	2010.7.3
加藤隆生、岩間達、鳥羽山寿子、鍵本聖一	腸重積の診断が困難であった14歳男児例	第 140 回日本小児科学会埼玉地方会	浦和	2010.9.11
鍵本聖一	ミトコンドリアとアポトーシス病態理解と治療のために	第 9 回浦安小児医療懇話会	浦安	2010.10.27
岩間達、鳥羽山寿子、萩原真一郎、鍵本聖一	小児腸管ベーチェット病 3 例の治療経験	第 11 回日本小児IBD 研究会	大阪	2011.2.13
萩原真一郎、岩間達、鳥羽山寿子、鍵本聖一	C-ANCA陽性の分類不能な腸炎の1例	第11回日本小児IBD研究会	大阪	2011.2.13

Ⅶ. 研究成果の印刷物・別刷

Transactivation of the dopamine receptor 3 gene by a single provirus integration results in development of B-cell lymphoma in transgenic mice generated from retrovirally transduced embryonic stem cells

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Gene transfer vectors based on retroviruses are commonly used in gene therapy applications because of their unique ability to integrate efficiently into host genomes. This ability also forms the basis of a transformation event that can be induced in transduced cells by transactivation of proto-oncogenes near the vector integration sites. Here, we report on the development of lymphoma in mice generated from embryonic stem cells transduced with an enhanced green fluorescent protein. The cells expressed B220, CD5, Mac1, and IgM on their surfaces and expressed transcription factors characteristic of B-cell lymphoma. Importantly, each mouse had a single copy of the provirus in its genome; the copy was integrated into the second intron of the dopamine receptor 3 (*D3*) gene, and high-level expression of D3 was detected only in the lymphoma cells. Ectopic expression of D3 in murine marrow cells resulted in preferential proliferation of cells at the pre–B-cell stage in response to a D3-specific agonist, but this proliferation was not observed in vivo. Cells cotransduced with D3 and Bcl-x_L genes had a phenotype similar to that of lymphoma in vivo, suggesting that the leukemogenesis induced by retroviral integration required "second hit" mutations of additional genes. (*Blood*. 2010;115(19):3930-3938)

Introduction

Gene therapy has the potential as an alternative form of therapy for diseases that are not amenable to conventional medical approaches. Various types of gene transfer methods, including the use of viral vectors, have been devised and tested in animal models and in gene therapy clinical trials. These clinical trials have proven that retroviral (including lentiviral) vectors are among the most effective gene transfer vehicles, especially for inherent genetic disorders.[1-4] This success is attributed mainly to the unique ability of retroviral vectors to integrate into the host DNA, which allows the stable presence of the transferred gene in the genome of the transduced cell and therefore promises to allow continued expression of the therapeutic genes. On the other hand, unwanted instances of retroviral gene transfer into hematopoietic stem cells, which is known as "genotoxicity of retroviral integration," have possibly occurred.[5,6] The most serious case is leukemogenesis by transactivation of genes neighboring the integration sites of the retroviral vectors in the genomes.[3,7,8] Indeed, in France and the United Kingdom, a considerable fraction of patients with X-linked severe combined immunodeficiency (X-SCID) diseases who received autologous $CD34^+$ cells genetically modified by the retroviral vectors to express the common γ-chain complementary DNA (cDNA) have developed T-cell leukemia, although almost all the patients recovered their immunologic function, and their clinical signs of the diseases were ameliorated by the gene therapy.[8,9]

The accepted explanation for mechanisms of leukemogenesis is that the retroviral vectors were integrated into the sites of host genomes near the proto-oncogenes such as *LMO2*, *CCND2*, or *Bmi1*, and this resulted in aberrant expression of the proto-oncogenes through T-cell differentiation, which caused malignant transformation.[7-9] Because some of the transformed cells harbored the multiple copies number of proviruses, however, little is known about the involvement of an integration event in leukemogenesis. In addition to abnormal expression of proto-oncogenes, some patients showed other chromosomal aberrances, which seemed to be the "second hits" in the course of tumorigenesis.[8,9]

Using an improved retroviral gene transfer system in which the viral promoter/enhancer regions are less susceptible to methylation in immature cells, including hematopoietic stem cells[10] and neural stem cells,[11] we have shown that stable expression of enhanced green fluorescent protein (EGFP) can be obtained in mice generated from embryonic stem (ES) cells transduced with EGFP cDNA.[12] Such chimeric mice had multiple provirus copies in their chromosomes. However, because each provirus was transmitted to their gametes independently, sequential mating with wild-type mice resulted in offspring with a single proviral integration.[12] Interestingly, one strain of mice with a single provirus integrated into the second intron of the dopamine receptor 3 (*D3*) gene developed B-cell lymphoma at approximately 1 year after birth. In the present study, we have attempted to elucidate the mechanism of leukemogenesis caused by retroviral integration in these mice.

Methods

Mice

C57BL/6N (B6) mice and Ly5.1 B6 mice were purchased from Nihon Clea, and nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice were purchased from Sankyo Lab Service. All experiments were

Submitted August 28, 2009; accepted January 22, 2010. Prepublished online as *Blood* First Edition paper, March 10, 2010; DOI 10.1182/blood-2009-08-240077.

The online version of this article contains a data supplement.

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approved by the Institutional Review Committee and performed in accordance with the guidelines of the University of Tsukuba.

Retroviral vector construction and preparation

Full-length mouse D3 cDNA was synthesized by polymerase chain reaction (PCR) of the 5′ part (472 bp) and the 3′ part (940 bp) using the total RNA extracted from Ly5.1 B6 brain, followed by ligation of the amplification fragments. Primer sets and PCR conditions used are shown in the supplemental Methods (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). An *Xho*I fragment containing the full-length D3 cDNA was cloned into GCDsap retroviral vector,[12] and an *Xho*I-*Cla*I fragment containing the internal ribosomal entry site and humanized Kusabira Orange (huKO)[10] was inserted into the vector (GCD/D3/huKO). We also constructed a vector to express the genes encoding Bcl-x_L[13] and truncated human nerve growth factor receptor (NGFR)[14] genes (GCD/Bcl-x_L/NGFR). All vectors were converted into the corresponding retroviruses by transduction into the packaging cell line 293gpg, as described.[10] The titers of retroviruses were 9.0×10^6 IU/mL on Jurkat cells.

Cell cultures

Lymphomatous B cells developed in transgenic mice were cultured in RPMI 1640 with 10% fetal calf serum (FCS; HyClone), 2mM L-glutamine, 100 U/mL penicillin G sodium, 100 μg/mL streptomycin sulfate, and 50 μg/mL 2-mercaptoethanol. 293gpg cells and the stromal cell line PA6 were maintained as previously described.[15,16]

For coculture experiments, 3×10^5 c-KIT$^+$/lineage$^-$ (KL) cells isolated from the bone marrow (BM) of Ly5.1 B6 mice were cultured on day 0 in StemPro34 (Invitrogen) in the presence of 50 ng/mL mouse stem cell factor, 100 ng/mL human thrombopoietin, and 10 ng/mL human Flt3-ligand (R&D Systems) in 24-well plates coated with human fibronectin fragment CH296 (RetroNectin; Takara Bio). On days 1 and 2, cells were infected with the recombinant retroviruses by adding 50 μL of the concentrated virus supernatants. At 2 hours after transduction, the supernatants were replaced with fresh media supplemented with cytokines. On day 3, the transduced KL cells (1×10^6) were cocultured in 6-well plates with PA6 cells in RPMI 1640 with 10% FCS in the presence of 20 ng/mL stem cell factor, 10 ng/mL mouse interleukin-7 (IL-7; R&D Systems), and 1nM D3-specific agonist 7-hydroxy-2-dipropylaminotetralin (DPAT; Alexis). Both floating and adherent cells were collected from the culture every 4 days and analyzed individually by cell counting and flow cytometry with a FACSCalibur (BD Biosciences).

For BM transplantation, 3×10^5 transduced KL cells were intravenously inoculated into Ly5.2 B6 mice that had been irradiated with 550 cGy twice with a 4-hour interval. Peripheral blood samples of recipients were analyzed every 4 weeks by cell counting and surface marker analysis.

In transplantation experiments with B lymphoma cells, 5×10^5 B lymphoma cells were inoculated into sublethally irradiated (250 cGy) NOD/SCID mice via the tail vein. The numbers of leukocytes in the peripheral blood of recipient mice were monitored every 4 weeks after transplantation. When the cell counts rose above 12 000 leukocytes/mL, splenic leukocytes obtained from the mice were intravenously inoculated into NOD/SCID mice that had been irradiated with 100 cGy.

All other culture reagents were purchased from Sigma-Aldrich.

Proliferation and apoptosis assay

Transduced KL cells were cultured on PA6 cells for 4 days. Pre-B cells (B220$^+$/Mac1$^-$), myeloid cells (B220$^-$/Mac1$^+$), or hematopoietic progenitor cells (CD43$^-$/c-KIT$^+$) were purified from floating cells, and pro-B cells (CD43$^+$/c-KIT$^-$) were purified from cultured adhered cells by using a FACSVantage (BD Biosciences) cell sorter. A total of 10 000 sorted cells were then cultured in RPMI 1640 with 10% FCS (HyClone) supplemented with 2-mercaptoethanol, 20 ng/mL stem cell factor, and 10 ng/mL mouse IL-7 in the presence of 1nM DPAT (Alexis) in 96-well plates. After 24 hours of incubation, 0.037 Mbq (1 μCi) of [methyl-^{3}H]-thymidine (GE Healthcare) was added to each well, and the mixture was incubated for a further 16 hours. Cultures were harvested with a Macro96 Cell Harvest (Molecular Devices), and the radioactivity in each well was measured with a liquid scintillation counter (LS6500; Beckman Coulter).

To assess the DNA fragmentation induced by apoptosis, genomic DNA isolated from B lymphoma cells and their derivatives transduced with the Bcl-x_L was treated with RNase and electrophoresed in a 1.5% agarose gel. For the viability assay, cells were stained with 1 mg/mL propidium iodide (Sigma-Aldrich) and analyzed with a FACSCalibur (BD Biosciences).

Morphologic analysis and karyotyping

Cytospin cell preparations were made on slide glasses and stained with a May-Gruenwald-Giemsa reagent (Merck). Samples were analyzed and images captured with an Axioplan 2 (Carl Zeiss). For karyotyping, B lymphoma cells were incubated in the presence of 0.02 mg/mL Colcemid (Sigma-Aldrich) for 30 minutes. After hypotonic treatment, cells were fixed with a solution of methanol–acetic acid (at a ratio of 3:1) solution. Giemsa-stained chromosome samples were analyzed and imaged under a DM2000 (Leica Microsystems), and the images were imported into the manufacturer's software as a series of .tif files.

Cell-surface analysis

The antibodies used were as follows: fluorescein isothiocyanate–conjugated anti–mouse B220 (RA3-6B2), c-KIT (2B8), CD45.1 (A20), and Mac1 (M1/70); phycoerythrin (PE)–conjugated anti–mouse CD4 (RM4-5), CD5 (53-7.3), CD8 (53-6.7), CD25 (PC61), and Mac1 (M1/70; BD Biosciences); c-KIT (2B8), immunoglobulin M (1B4B1), IL-7Ra (A7R34), and Ter-119 (eBioscience); biotinylated anti–mouse CD5 (53-7.3), CD24 (30-F1), CD43 (S7), and streptavidin-PE (BD Biosciences), CD40 (1C10, HM40-3), CD80 (16-10A1), CD86 (GL1), c-KIT (2B8), and MHC class II (M5/114.15.2; eBioscience); PE-Cy5–conjugated anti–mouse CD3 (145-2C11), B220 (RA3-6B2), Mac1 (M1/70), streptavidin–PE-Cy5, and allophycocyanin-conjugated anti–mouse B220 (BioLegend); and anti–human NGFR (Miltenyi Biotec). Cells were stained with antibodies after lysis of red blood cells and staining with anti–mouse CD16/32 (93; Beckman Coulter) for Fc receptor blocking. This was followed by analysis with a FACSCalibur (BD Biosciences).

PCR

Reverse transcription–PCR (RT-PCR) was used to analyze expression of transcription factors related to B-cell proliferation/differentiation in splenic B cells purified from B6 mice by negative selection using biotinylated anti-mouse CD4, CD8, Gr-1, Mac1, and Ter-119 antibodies and streptavidin-magnetic beads (MyOne Streptavidin C1; Invitrogen) and in B lymphoma cells. For quantitative RT-PCR, 1 μg of the first-strand cDNA prepared from splenic B cells or B lymphoma cells was mixed with mouse D3 or β-actin TaqMan MGB probe, each primer set, and TaqMan Universal PCR Master Mix and assayed in an ABI PRISM 7900HT (Applied Biosystems). For clonality analysis, genomic DNA was isolated from splenic B cells or B lymphoma cells using SepaGene (Sanko Junyaku). After incubation of the genomic DNA with RNase (Sigma-Aldrich), 100 ng of the DNA was used for analysis of V(D)J rearrangement. To assess the integration sites, linear amplification–mediated PCR (LAM-PCR) was performed as previously described,[17] with some modifications. PCR products cloned by LAM-PCR were sequenced, and the genomic coordinates of the integration sites were determined by interrogation of the mouse genome database of the National Center for Biotechnology Information (NCBI).[18] Primer sets and PCR conditions used in these experiments are shown in supplemental Methods.

Southern and Northern blot analyses

A total of 20 μg of genomic DNA obtained from the tails of mice was digested with *Eco*RI or *Bam*HI (Takara Bio). The membrane to which the DNA was transferred (Biodyne Nylon Membrane; Pall) was hybridized to deoxycytidine [α-^{32}P] (GE Healthcare)–labeled EGFP and D3 probes. The *Bam*HI cuts once within the vector sequence: therefore, the number of

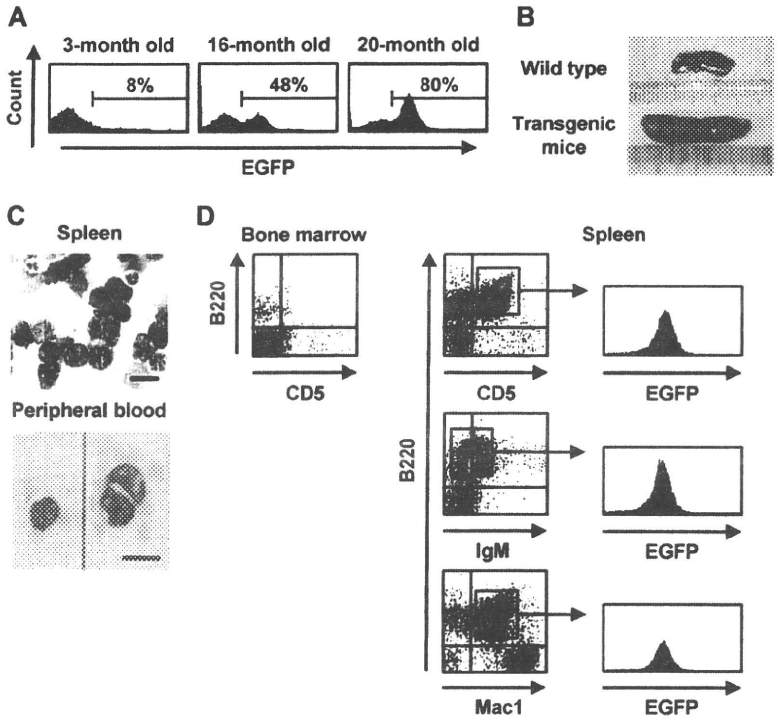

Figure 1. Hematologic analysis of the transgenic mice affected with B lymphoma. (A) EGFP expression in peripheral blood of a transgenic mouse affected with typical hematologic abnormalities at the indicated ages. (B) Spleens of 20-month-old wild-type and transgenic mice. (C-D) Morphologic appearance of spleen and peripheral blood cells (May-Gruenwald-Giemsa staining 63×/1.4 NA oil objective; C) and flow cytometric analysis of bone marrow and spleen cells (D) of transgenic mice exhibiting remarkable expansion of EGFP$^+$ cells. Bars represent 10 μm in panel C.

fragments hybridized with the EGFP probe corresponds to the number of proviruses integrated into the host genome. Hybridization and the following steps were performed in Perfect Hyb buffer (TOYOBO) in accordance with the manufacturer's instructions. For Northern blotting, 30 μg of total RNA obtained from the brains of B6 mice or B-lymphoma cells was pretreated with 3-[N-morpholino] propanesulfonic acid buffer, formaldehyde, and formamide (Sigma-Aldrich), then electrophoresed in a 1.8% agarose gel. The transferred membrane (Hybond-N+; GE Healthcare) was hybridized to deoxycytidine [α-^{32}P]–labeled D3 probes. Hybridization and the following steps were performed in ULTRAhyb Ultrasensitive Buffer (Applied Biosystems) in accordance with the manufacturer's instructions.

Microarray-based gene expression profiling

An AllPrep Mini kit (QIAGEN) was used to extract total RNA from 2 cell lines that were independently established from mice developing lymphoma (L1 and L2) and from splenocytes from 2 B6 mice as controls (S1 and S2). A detailed protocol of the microarray analysis is given in the supplemental Methods. Briefly, labeled complementary RNA was prepared from the total RNA by using the Agilent labeling protocol (Agilent Technologies). Genes/transcripts were considered to be up- or down-regulated in the lymphoma compared with normal splenocytes if the normalized signal values of the genes/transcripts were more than 100 in each sample, and if the fold-change values were consistently higher than 2.0 or lower than 0.5. Gene Ontology analysis for the differentially expressed genes was conducted by using the DAVID Web site.[19,20] All microarray data may be found on the Gene Expression Omnibus (GEO) public database under accession number GSE20661 (National Center for Biotechnology Information [NCBI], http://www.ncbi.nlm.nih.gov, submitted March 5, 2010).

Statistical analysis

Kaplan-Meier estimation with the SAS-type log-rank test was used for survival analysis. All other statistical analyses were performed with the Mann-Whitney *U* test.

Results

Development of B-cell lymphoma in transgenic mice

We used the retroviral vector GCDsap to generate transgenic mice from ES cells that were retrovirally transduced with EGFP. These mice demonstrated stable expression of the gene and the ability to transfer this feature to their progeny, although the proportion of EGFP-expressing cells and the mean intensity of expression varied, even in siblings that harbored proviruses integrated into the same chromosome sites, suggesting that gene silencing of the vector occurred in a discontinuous and stochastic manner during cell division.[21] Interestingly, approximately half of the F1 mice with EGFP expression that were born from a chimeric mouse (9 of 19 mice) showed some sort of hematologic abnormalities, such as splenomegaly and the appearance of CD5$^+$B220$^+$ cells in their peripheral blood, and they showed a gradual increase of the proportion of EGFP$^+$ cells in their peripheral blood with age. Typically, the percentage of EGFP$^+$ cells rose from 8% at the age of 3 months to up to 80% at 20 months after birth (Figure 1A). Concomitant with the increase in the percentage of EGFP$^+$ cells,

Table 1. Expression of surface antigens on B-lymphoma cells

	CD5	CD24	CD25	CD40	CD43	CD80	CD86	c-KIT	IgM	MHC II
Splenic B cell, %	21	78	0.7	32	13	59	57	4.1	92	99
B-lymphoma cell, %	99	74	1.4	19	88	85	95	1.2	76	94

Mean percentages of surface antigens on splenic B cells obtained from wild-type mice or B-lymphoma cells generated from EGFP-transgenic mice are shown.