45:213-22, 2007.

Yamada M, Tanemura K, Ozawa M, Ohyama R, Kitamura N, Kawano M, Tan-Takeuchi K, Mizuno H, Okada S, Iwanami A, Nakamura M, Ishitsuka C, Nagai A, Miyawaki A, Takashima A, Ogawa M, Okano H, Kondoh T: Electrical stimulation modulates fate determination of differentiating embryonic stem cells. Stem Cells in press.

Kato N, Shimmura S, Kawakita T, Miyashita H, Ozawa Y, Yoshida S, Higa K, Okano H, Tsubota K: Beta-catenin activation and epithelial-mesenchymal transition in the pathogenesis of pterygium. IOVS in press

Okano H: Adult neural stem cells and central nervous system repair. Ernst Schering Res Found Workshop. 60: 215-28, 2006.

Yoshida S, Shimmura S, Nagoshi N, Matzuzaki Y, Fukuda K, Okano H, Tsubota K: Isolation of multipotent neural crest-derived stem cells from the adult cornea. Stem Cells 12:2714-22, 2006.

Ishii K, Nakamura M, Dai HN, Finn TP, Okano H, Toyama Y, Bregman BS: Neutralization of Ciliary Neurotrophic Factor reduces astrocyte production from transplanted neural stem cells and promotes regeneration of corticospinal tract fibers in spinal cord injury. J Neurosci Res 84:1669-81, 2006.

Kurihara T, Ozawa Y, Shinoda K, Nagai N, Inoue M, Oike Y, Tsubota K, Ishida S, Okano H: Role of the angiotensin II type 1 receptor (AT1R) blocker, telmisartan, in neuroprotection against retinal inflammation. IOVS 47:545-52, 2006.

Arata Y, Kouike H, Zhang Y, Herman M, Okano H, Sawa H: Wnt signaling and a Hox protein cooperatively regulate PSA-3/MEIS to determine daughter cell fate after asymmetric cell division in C. elegans. Dev Cell 11:105-15, 2006.

Okada S, Ishii K, Miyao T, Shimzakai T, Katoh H, Yamane J, Yoshimura A, Iwamoto Y, Nakamura M, Toyama Y, Okano H: Conditional ablation of STAT3/SOCS3 discloses a

dual role for reactive astrocytes after spinal cord injury. Nat Med 12: 829-34, 2006.

Chan C, Moore BE, Cotman CW, Okano H, Tavares R, Hovanesian V, Pinar H, Johanson CE, Svendsen CN, Stopa EG: Musashi1 antigen expression in human fetal germinal matrix development. Exp. Neurol 201: 515-8, 2006.

Ninomiya M, Yamashita T, Araki N, Okano H, Sawamoto K: Enhanced neurogenesis in the ischemic striatum following EGF-induced expansion of transit-amplifying precursors in the subventricular zone. Neurosci Lett 403: 63-7, 2006.

Yamashita T, Ninomiya M, Acosta PH, García-Verdugo JM, Sunabori T, Sakaguchi M, Adachi K, Kojima T, Hirota Y, Kawase T, Araki N, Abe K, Okano H, Sawamoto K: Subventricular-zone-derived neuroblasts migrate and differentiate into mature neurons in the post-stroke adult striatum. J Neurosci 26: 6627-36, 2006.

Sakaguchi M, Shingo T, Shimazaki T, Okano HJ, Shiwa M, Ishibashi S, Oguro H, Ninomiya M, Kadoya T, Horie H, Shibuya A, Mizusawa H, Poirier F, Nakauchi H, Sawamoto K, Okano H: A carbohydrate binding protein, Galectin-1, promotes proliferation of adult neural stem cells. Proc Natl Acad Sci USA 103:7112-7, 2006.

Hara T, Nakamura K, Nakahara Y, Migishima R, Yokoyama M, Okano H, Mizushima N: Suppression of autophagy in neural cells causes a neurodegenerative disease. Nature 441: 885-9, 2006.

Miyagi S, Nishimoto M, Saito T, Ninomiya M, Sawamoto K, Okano H, Muramatsu M, Oguro H, Iwama A, Okuda A: The Sox2 regulatory region 2 functions as a neural stem cell specific enhancer in the telencephalon. J Biol Chem 281:13374-81, 2006.

Artaamangkul S, Torrecilla M, Kobayashi K, Okano H, Williams JT: Separation of Mu opioid receptor desensitization and internalization. J Neurosci 26:4118-25, 2006.

Rawal N, Castelo-Branco G, Sousa KM, Kobayashi K, Okano H, Arenas E: Spatial and temporal expression dynamics of Wnt signaling components in the developing midbrain.

Exp Cell Res 312:1626-36, 2006.

Murata J, Tokunaga A, Okano H, Kubo T: Notch1 activation demonstrated in situ, during mouse cochlear development. J Comp Neurol 497:502-18, 2006.

Tonchev AB, Yamashima T, Sawamoto K, Okano H: Transcription factor protein expression patterns by neural or neuronal progenitor cells of adult monkey subventricular zone. Neuroscience 139:1355-67, 2006.

Okano H: Thoughts on the biomedical sciences in Japan and Australia. Inflammation and Regeneration. 26:16-7, 2006.

Sawamoto K, Wichterle H, Gonzalez-Perez O, Cholfin JA, Yamada M, Spassky N, Murcia NS, Garcia-Verdugo JM, Martin O, Rubenstein JL, Tessier-Lavigne M, Okano H, Alvarez-Buylla A: New neurons follow the flow of cerebrospinal fluid in the adult brain. Science 311:629-31, 2006.

Matsumoto A, Okada Y, Nakamichi M, Nakamura M, Aoki M, Sobue G, Itoyama Y, Okano H: Disease progression of SOD1 (G93A) ALS model rats. J Neurosci Res 83: 119-33, 2006.

Takenaga M, Ohta Y, Tokura Y, Hamaguchi A, Nakamura M, Okano H, Igarashi R: Lecithinized superoxide dismutase (PC-SOD) improved spinal cord injury-induced motor dysfunction through suppression of oxidative stress and enhancement of neurotrophic factor production. J Control Release 110:283-9, 2006.

Nagata H, Akiba Y, Suzuki H, Okano H, Hibi T: Expression of Musashi-1 in the rat stomach and changes during mucosal injury and reconstitution. FEBS Lett 580:27-33, 2006.

Kanai R, Tomita H, Shinoda A, Takahashi M, Goldman SA, Okano H, Kawase T, Yazaki T: Enhanced therapeutic efficacy of G207 for the treatment of glioma through Musashi1 promoter retargeting of gamma 34.5-mediated virulence. Gene Therapy 13:

106-16, 2006.

Fujioka M, Kanzaki S, Okano HJ, Masuda M, Ogawa K, Okano H: Proinflammatory cytokines expression in noise-induced damaged cochlea. J Neurosci Res 83: 575-83, 2006.

Kawada H, Takizawa S, Takanashi T, Morita Y, Fujita J, Fukuda K, Takagi S, Okano H, Ando K, Hotta T: Administration of hematopoietic cytokines in the subacute phase after cerebral infarction is effective for functional recovery facilitating proliferation of intrinsic neural stem cells and transition of bone marrow-derived neuronal cells. Circulation 113:701-10, 2006.

Nishimura T, Yamaguchi T, Tokunaga A, Hara A, Hamaguchi T, Kato K, Iwamatsu A, Okano H, Kaibuchi K: Role of Numb in dendritic spine development with a Cdc42 GEF intersectin and EphB2. Mol. Biol. Cell 17:1273-85, 2006.

Toriya M, Tokunaga A, Sawamoto K, Nakao K, Okano H: Distinct functions of human Numb isoforms revealed by misexpression in neural precursor cells in Drosophila larval brain. Dev. Neurosci 28:142-55, 2006.

Sugiyama-Nakagiri Y, Akiyama M, Shibata S, Okano H, Shimizu H: Expression of RNA-binding protein Musashi in hair follicle development and hair cycle progression. Am J Pathol 168:80-92, 2006.

Fukuhara Y, Li X-K, Kitazawa Y, Inagaki M, Matsuoka K, Kosuga M, Kosaki R, Shimazaki T, Endo H, Umezawa A, Okano H, Takahashi T, Okuyama T: Histopathological and behavioral improvement of murine Mucopolysaccharidosis type VII by intra-cerebral transplantation of neural stem cells. Molecular Therapy 13: 548-55, 2006.

Okano H, Nakamura M, Toyama Y: Stem cell therapies for injured spinal cord. Inflammation and Regeneration 26:18-28, 2006.

Okano H, Kohyama J, Ohba H, Sakaguchi M, Tokunaga A, Shimazaki T, Okano HJ: Neural stem cells: Isolation and self-renewal. In Tissue Stem Cells; Biology & Applications. (Edited by Potten , Wilson, Clarke, Renehan, Marcel Dekker), Taylor & Francis Group, LLC, New York, pp. 55-70, 2006.

Okano H: Transplantation of neural stem cells for spinal cord regeneration. In Encyclopedic References of Neuroscience (Edited by Binder D, Hirokawa N and Windhorst U) Springer-Verlag (Heidelberg, Germany), 2006.

Matsumoto A, Okada Y, Nakamichi M, Nakamura M, Aoki M, Sobue G, Itoyama Y, Okano H: Disease progression of SOD1 (G93A) ALS model rats. J Neurosci Res 83:119-33, 2006.

分担研究者：飯島正文

Nakada T, Akiyama M, Iijima M, Kato A, Maibach HI: Drug eruptions to contrast media in Japan. Clin Exp Dermatol 31:361-364, 2006.

Sagara R, Kitami A, Nakada T, Iijima M: Adverse reactions to gefitinib (Iressa®): Revealing sycosis - and pyoderma gangrenosum-like lesions. Int J Dermatol 45:1002, 2006

Matsumura T, Watanabe H, Batchelor J, Sueki H, Iijima M: Drug eruption caused by the nonionic contrast medium iohexol. ”Recall-like phenomenon” appearing on an area previously affected by herpes zoster. J Dermatol 33:705-8, 2006

中田土起丈, 飯島正文:薬疹患者へのパッチテスト結果. 医薬の門 46:341-343, 2006

末木博彦, 飯島正文：SJS/TEN の早期診断と治療 −皮膚科医の役割−. 皮膚アレルギーフロンティア 4：73-77, 2006

分担研究者：池澤善郎

Inomata N, Osuna H, Fujita H, Ogawa T, Ikezawa Z: Multiple chemical sensitivities following intolerance to azo dye in sweets in a 5-year-old girl. Allergol Int. 55:203-5, 2006.

Minami M, Ikezawa Z.: Effects of Tributyltin on the Development of Atopic Dermatitis-Like Eruptions in DS-Nh Mice. Int Arch Allergy Immunol 141:337-45, 2006

Mitani N, Nagatani T, Ikezawa Z, Kakemizu N, Yamakawa Y, Aihara M, Nozawa A, Tomita N, Tanaka K.: A case of cutaneous T cell pseudolymphoma in a patient with Helicobacter pylori infection. Dermatology 213:156-8, 2006

Kambara T, Aihara M, Matsukura S, Sato I, Kubota Y, Hirasawa T, Ikezawa Z.: Effects of photocatalytic agent on DS-Nh mice, developing atopic dermatitis-like eruption with an increase of Staphylococcus aureus. Int Arch Allergy Immunol 141:151-7, 2006

西　香織、山野朋子、山川有子、相原道子、池澤善郎：イソプロピルアンチピリンによるアナフィラキシーの 1 例、皮膚臨床科、48：590-591, 2006.

山野朋子, 山川有子, 相原道子, 池澤善郎：分子標的治療薬ゲフィチニブおよびイマチニブによる皮膚病変の検討：臨床皮膚科　60:771-775, 2006.

池澤善郎：薬疹　?. 膠原病・免疫・アレルギー. 内科　増大号, 97:1242-1244, 2006,6.
池澤善郎：薬剤アレルギーのための負荷テスト. 小児内科,　38:1436-1439, 2006.

山根裕美子, 池澤善郎：抗菌薬の皮内テストのエビデンス. 皮膚アレルギーフロンティア, 4:114-117, 2006.

山根裕美子, 相原道子, 池澤善郎：薬疹とアレルギー. 臨牀看護, 32:1478-1483, 2006.

池澤善郎：6.中毒性表皮壊死融解症(TEN). A.これだけは知っておくべき薬疹の基礎知識　1.薬疹の分類, 皮膚科診療プラクティス　19 薬疹を極める（塩原哲夫, 宮地良樹, 瀧川雅浩編）, 49－53, 文光堂, 東京, 2006.

池澤善郎：重症薬疹の病型・診断・治療. 第 10 章その他の非腫瘍性皮膚疾患, 皮膚疾患の最新医療（斎田俊明, 飯塚　一, 清水　宏, 竹原和彦, 古江増隆, 池田志斈, 石川　治, 玉井克人編）, 208－212, 先端医療技術研究所, 東京, 2006.

池澤善郎：106 中毒性表皮壊死症の診断と治療指針.環境障害・電解質異常・皮膚障害の診断・治療・ケア, 救急・集中治療ガイドライン一最新の診療指針一(岡元和文編), 799－801,総合医学社, 東京, 2006.

池澤善郎：1.薬疹の頻度, 2.薬疹の臨床, 3.薬物アレルギー機序と薬疹の発疹型, 4.薬疹にみる薬物アレルギーの交差反応　コラム 1：重症薬疹とその診断基準　コラム 2：薬疹とウィルス感染,とくに伝染性単核球症のアンピシリン疹と drug-induced hypersensitivity syndrome(DIHS)について.　薬物アレルギー, 薬物療法学(石崎高志, 鎌滝哲也, 望月眞弓編), 77－86, 南江堂, (東京), 2006.

山野朋子, 相原道子, 池澤善郎：3 イレッサR・グリベックRによる薬疹. 薬疹, WHAT'S NEW in 皮膚科学(宮地良樹編), 64－65, メディカルレビュー社, 東京, 2006.

分担研究者：塩原哲夫

Shiohara T, Inaoka M, Kano Y: Drug-induced hypersensitivity syndrome (DIHS): A reaction induced by a complex interplay among herpesviruses and antiviral and antidrug immune responses. Allergol Int 55:1-8, 2006.

Kano Y, Hirahara K, Sakuma K, Shiohara T: Several herpesviruses can reactivate in a severe drug-induced multiorgan reaction in the same sequential order as in graft-versus-host disease. Br J Dermatol. 155:301-306, 2006.

Kano Y, Seishima M, Shiohara T: Letter. Hypogammaglobulinemia as an early sign of drug-induced hypersensitivity syndrome. J Am Acad Dermatol 55:727-728, 2006.

武林亮子, 寺木祐一, 塩原哲夫: 1 年を隔ててフェニトインとカルバマゼピンにより生じた非典型drug-induced hypersensitivity syndrome−DLSTの興味ある結果を加えて−. 皮膚臨床 48(1): 45-48, 2006.

塩原哲夫: III.診療現場で必ず役立つ知識. 2.薬疹の見分け方. 臨床研修プラクティス 3(3): 60-62, 2006.

塩原哲夫: 薬疹. ガイドライン外来診療2006. 泉 孝英編. 東京, 日経メディカル, 2006.3, p.256-260.

塩原哲夫: 変貌する薬疹の概念. 皮膚科診療プラクティス 19 薬疹を極める. 塩原哲夫編, 東京, 文光堂, 2006. p.2-9.

塩原哲夫: 薬疹を疑う患者さんが来られたら−いかに本書を活用するか−. 皮膚科診療プラクティス 19 薬疹を極める. 塩原哲夫編, 東京, 東京, 文光堂, 2006. p.18-22.

塩原哲夫: 間擦疹型薬疹. 皮膚科診療プラクティス 19 薬疹を極める. 塩原哲夫編, 東京, 文光堂, 2006. p.54-58.

塩原哲夫: 薬疹とCRP. 皮膚科診療プラクティス 19 薬疹を極める. 塩原哲夫編, 東京, 文光堂, 2006. p.132-133.

塩原哲夫: 薬剤誘発性皮膚疾患をどのように考えるか？ 皮膚科診療プラクティス 19 薬疹を極める. 塩原哲夫編, 東京, 文光堂, 2006. p.150-155.

塩原哲夫: 免疫再構築症候群とは. 皮膚科診療プラクティス 19 薬疹を極める. 塩原哲夫編, 東京, 文光堂, 2006. p.252-253.

分担研究者：木下　茂

Kinoshita S. Comparison of intact and denuded amniotic membrane as a substrate for cell-suspension culture of human limbal epithelial cells. Graefes Arch Clin Exp Ophthalmol in press.

NakamuraT, Kinoshita S: Novel Clinical application of sterilized, freeze-dried amniotic membrane to treat patients with pterygium. Acta Ophthalmologica Scand in press.

Inatomi T, Nakamura T, Kojyo M, Koizumi N, Sotozono C, Kinoshita S: Ocular surface reconstruction with combination of cultivated oral mucosal epithelial transplantation and penetrating keratoplasty. Am J Ophthalmol 142:757-64, 2006.

Ang LPK, Sotozono C, Koizumi N, Suzuki T, Inatomi T, Kinoshita S: A comparison between cultivated and conventional limbal stem cell transplantation for Stevens-Johnson syndrome. Am J Ophthalmol 143:178-80, 2007.

Kaido M, Dogru M, Yamada M, Sotozono C, Kinoshita S, Shimazaki J, Tsubota K: Functional visual acuity in Stevens-Johnson syndrome. Am J Ophthalmol 142:917-22, 2006.

Ueta M, Sotozono C, Tokunaga K, Yabe T, Kinoshita S: Strong association between HLA-A*0206 and Stevens-Johnson syndrome in the Japanese. Am J Ophthalmol 143:367-8, 2007.

Ueta M, Iida T, Sakamoto M, Sotozono C, Takahashi J, Kojima K, Okada K, Xiuhao C, Kinoshita S, Honda T: Polyclonality of Staphylococcus epidermidis residing on the healthy ocular surface. J Med Microbiol 56:77-82, 2007.

Matsuda A, Kumagai N, Fukuda K, Ebe K, Hirano K, Sotozono C, Tei M, Hasagawa K, Shimizu M, Tamari M, Ohno S, Mizuki N, Ikezawa Z, Shirakawa T, Hamuro J, Kinoshita S: Functional SNPs in the promoter of the IFNGR1 gene are associated with

ocular complications of atopic dermatitis. IOVS in press.

Sotozono C, Ang LPK, Koizumi N, Higashihara H, Ueta M, Inatomi T, Yokoi N, Kaido M, Murato D, Shimazaki J, Tsubota K, Yamada M, Kinoshita S: ,A new grading system for the evaluation of chronic ocular manifestations in patients with Stevens-Johnson syndrome. Ophthalmology in press.

Nakamura T, Inatomi T, Cooper LJ, Rigby H, Fullwood NJ, Sotozono C, Kinoshita S. Phenotypic investigation of human eyes with transplanted autologous cultivated oral mucosal epithelial sheets for severe ocular surface diseases. Ophthalmology in press.

Connon CJ, Kawasaki S, Liles M, Koizumi N, Yamasaki K, Nakamura T, Quantock AJ, Kinoshita S: Gene expression and immunolocalization of a calcium activated chloride channel during the stratification of cultivated and developing corneal epithelium. Cell Tissue Res 323:177-82, 2006.

Cooper LJ, Kinoshita S, German M, Koizumi N, Nakamura T, Fullwood NJ: An investigation into the composition of amniotic membrane used for ocular surface reconstruction. Cornea 24: 722-9, 2005.

Connon CJ, Nakamura T, Quantock AJ, Kinoshita S: The persistence of transplanted amniotic membrane in corneal stroma. Am J Ophthalmol 141:190-2, 2006.

Inatomi T, Nakamura T, Koizumi N, Sotozono C, Yokoi N, Kinoshita S: Mid-term results on ocular surface reconstruction using cultivated autologous oral mucosal epithelial transplantation. Am J Ophthalmol 141:267-75, 2006.

Koizumi N, Yamasaki K, Kawasaki S, Sotozono C, Inatomi T, Mochida C, Kinoshita S: Cytomegalovirus in aqueous humor from an eye with corneal endothliitis. Am J Ophthalmol 141:564-5, 2006.

Nakamura T, Ang LPK, Rigby H, Sekiyama E, Inatomi T, Sotozono C, Fullwood NJ, Kinoshita S: The use of autologous serum in the development of corneal and oral

epithelial equivalents in patients with Stevens Johnson syndrome. Invest Ophthalmol Vis Sci 47:909-16, 2006.

Sekiyama E, Nakamura T, Cooper LJ, Kawasaki S, Hamuro J, Fullwood NJ, Kinoshita S: Unique distribution of thrombospondin-1 in human ocular surface epithelium. Invest Ophthalmol Vis Sci 47:1352-8, 2006.

Kawasaki S, Tanioka H, Yamasaki K, Yokoi N, Komuro A, Kinoshita S: Clusters of corneal epithelial cells reside ectopically in human conjunctival epithelium. Invest Ophthalmol Vis Sci 47:1359-67, 2006.

Watanabe A, Araki B, Noso K, Kakizaki H, Kinoshita S: Histopathology of blepharoptosis induced by prolonged hard contact lens wear. Am J Ophthalmol 141:1092-6, 2006.

Koizumi N, Rigby H, Fullwood, NJ, Kawasaki S, Tanioka H, Koizumi K, Kociok N, Joussen AM, Hozono Y, Ueta M, Hamuro J, Kojima K, Kawasaki S, Yamazaki K, Kinoshita S: Human corneal epithelial cells respond to ocular-pathogenic, but not to nonpathogenic- flagellin. BBRC 347:238-47, 2006.

Sekiyama E, Nakamura T, Kawasaki S, Sogabe H, Kinoshita S: Different expression of angiogenesis-related factors between human cultivated corneal- and oral epithelial sheet. Exp Eye Res 83:741-6, 2006.

Nakamura T, Inatomi T, Sotozono C, Ang LPK, Koizumi N, Yokoi N, Kinoshita S: The transplantation of autologous serum-derived cultivated corneal equivalents for the treatment of severe ocular surface disease. Ophthalmology 113:1765-72, 2006.

Ang LPK, Nakamura T, Inatomi T, Sotozono C, Koizumi N, Yokoi N, Kinoshita S: Autologous serum-derived cultivated oral epithelial transplantation for severe ocular surface disease. Arch Ophthalmol 124:1543-51, 2006.

Ueno M, Matsumura M, Watanabe K, Nakamura T, Osakada F, Takahashi M, Kawasaki

H, Kinoshita S, Sasai Y: Neural conversion of embryonic stem cells by an inductive activity on human amniotic membrane matrix. PNAS 103: 9554-9, 2006.

Tanioka H, Kawasaki S, Yamasaki K, Ang LPK, Koizumi N, Nakamura T, Yokoi N, Komuro A, Inatomi T, Kinoshita S: Establishment of a human conjunctival epithelium as an alternative tissue source for autologous corneal epithelial transplantation. Invest Ophthalmol Vis Sci 47:3820-7, 2006.

Koizumi N, Kinoshita S: Postoperative management of lamellar keratoplasty: optical and tectonic. In: John T, ed. Surgical Techniques in Anterior and Posterior Lamellar Corneal Surgery, New Delhi, Jaypee Brothers, pp. 654-62, 2006.

分担研究者：森田栄伸

Zhang Y, Matsuo H, Morita E: Cross-reactivity among shrimp, crab and scallops in a patient with a seafood allergy. J Dermatol 33:174-7, 2006

Zhang Y, Matsuo H, Morita E: Increased production of vascular endothelial growth factor in the lesions of atopic dermatitis. Arch Dermatol Res 297:425-9, 2006

分担研究者：相原雄幸

Aihara Y, Ito R, Ito S, Aihara M, Yokota S: Successful treatment of toxic epidermal necrolysis in a child treated with cyclosporin A and methylprednisolone. Pediatr Int in press.

Ichikawa K, Ito R, Kobayashi Y, Aihara M, Osuna H, Aihara Y: A pediatric case of anaphylaxis and chemical mediators' levels caused by Matsutake mushroom (Tricholoma matsutake) ingestion. Allergol Int 55:85-8, 2006

Ito S, Anze M, Ichikawa K, Aihara Y, Yokota S: Kawasaki disease after burn. Eur J

Pediatr 165:340-1, 2006

Ito S, Takeshita S, Nezu A, Aihara Y, Usuku S, Nogichi Y, Yokota S: Norovirus-associated encephalopathy. Pediatr Inf Dis J 25:651-2, 2006

Ito S, Nakamura T, Kurosawa R, Miyamae T, Imagawa T, Mori M, Aihara Y, Yokota S: Glomerulonephritis in children with mixed connective tissue disease. Clin Nephrol 66:160-5, 2006.

Harada T, Machida H, Ito S, Aihara Y, Yokota S. Henoch-Schonlein purpura presenting duodenal involvement similar to superior mesenteric artery syndrome in a girl. Eur J Pediatr in press.

黒澤るみ子、梅林宏明、今川智之、片倉茂樹、森　雅亮、満田年宏、相原雄幸、横田俊平：小児期発症全身性エリテマトーデス 38 例の臨床的特徴と診断における問題点 日本小児科学会誌 110:398-405,2006

相原雄幸：若年性特発性関節炎（多関節型）小児科診療 69 増刊号 302-305,2006

相原雄幸：マイコプラズマ感染症と Stevens-Johnson 症候群　皮膚科診療プラクテイス 19　薬疹を極める　塩原哲夫編集　文光堂　2006 年 5 月 p 235-239

相原雄幸：小児アレルギー学の新しい展開 食物依存性運動誘発アナフィラキシーの診断と対処法.小児科臨床増刊　　59：1394-1402,2006

分担研究者：玉井克人

Kaneko T, Tamai K, Matsuzaki Y, Yamazaki T, Nakano H, Kon A, Hashimoto I, Hanada K, Kaneda Y, Uitto J: Interferon-gamma down-regulates expression of the 230-kDa bullous pemphigoid antigen gene (BPAG1) in epidermal keratinocytes via novel chimeric sequences of ISRE and GAS. Exp Dermatol 15:308-14, 2006.

Nishikawa T, Nakagami H, Matsuki A, Maeda A, Yo CY, Harada T, Morishita R, Tamai K, Kaneda Y: Development of High-Throughput Functional Screening of Therapeutic Genes, Using a Hemagglutinating Virus of Japan Envelope Vector. Hum Gene Ther 17:470-5, 2006.

Kunugiza Y, Tomita N, Taniyama Y, Tomita T, Osako MK, Tamai K, Tanabe T, Kaneda Y, Yoshikawa H, Morishita R: Acceleration of wound healing by combined gene transfer of hepatocyte growth factor and prostacyclin synthase with Shima Jet. Gene Ther 13:1143-52, 2006.

Mima H, Yamamoto S, Ito M, Tomoshige R, Tabata Y, Tamai K, Kaneda Y: Targeted chemotherapy against intraperitoneally disseminated colon carcinoma using a cationized gelatin-conjugated HVJ envelope vector. Mol Cancer Ther 5:1021-8, 2006.

Saito Y, Nakagami H, Morishita R, Takami Y, Kikuchi Y, Hayashi H, Nishikawa T, Tamai K, Azuma N, Sasajima T, Kaneda Y: Transfection of human hepatocyte growth factor gene ameliorates secondary lymphedema via promotion of lymphangiogenesis. Circulation. 114:1177-84, 2006.

Yasuoka E, Oshima K, Tamai K, Kubo T, Kaneda Y: Needleless intranasal administration of HVJ-E containing allergen attenuates experimental allergic rhinitis. J Mol Med in press.

Iwata T, Nakano H, Nakano A, Toyomaki Y, Tamai K, Tomita Y: Dominant dystrophic epidermolysis bullosa caused by a novel G2037R mutation and by a known G2028R mutation in the type VII collagen gene (COL7A1). J Dermatol 33:550-6, 2006.

玉井克人：NFkB デコイ DNA 軟膏によるアトピー性皮膚炎治療、感染、炎症、免疫　2006; 36: 238-240.

玉井克人：基底膜領域の生化学、片山一朗、土田哲也、橋本隆、古江増隆、渡辺晋一編、皮膚科学、東京：文光道:2006. p.37-39.

玉井克人：遺伝性皮膚疾患の遺伝子治療、斎田俊明、飯塚一、清水宏、竹原和彦、古江増隆、池田志学、石川治、玉井克人編、先端医療シリーズ第38巻、皮膚科：皮膚疾患の最新医療、東京;先端医療技術研究所:2006. p.29-33.

分担研究者：岸本治郎

Soma T, Ishimatsu-Tsuji Y, Tajima M, Kishimoto J: Runx1 transcription factor is involved in the regulation of KAP5 gene expression in human hair follicles. J Dermatol Sci 41:221-4, 2006.

Shirakata Y, Kishimoto J, Tokumaru S, Yamasaki K, Hanakawa Y, Tohyama M, Sayama K, Hashimoto K: Epiregulin, a member of the EGF family, is over-expressed in psoriatic epidermis. J Dermatol Sci 45:69-72, 2007.

Soma T, Yano K, Kishimoto J: Biotechnology in hair care.(II): Growth modulation. in Cosmetic science and technology series 29, 267-296, Taylor & Francis, New York 2006.

分担研究者：白方裕司

Shirakata Y, Kishimoto J, Tokumaru S, Yamasaki K, Hanakawa Y, Tohyama M, Sayama K, Hashimoto K.: Epiregulin, a member of the EGF family, is over-expressed in psoriatic epidermis. J Dermatol Sci 45:69-72, 2007

Shiraishi K, Yamasaki K, Nanba D, Inoue H, Hanakawa Y, Shirakata Y, Hashimoto K, Higashiyama S.: Pre-B-cell leukemia transcription factor 1 is a major target of promyelocytic leukemia zinc-finger-mediated melanoma cell growth suppression. Oncogene 26:339-48, 2007

Yang L, Yamasaki K, Shirakata Y, Dai X, Tokumaru S, Yahata Y, Tohyama M, Hanakawa Y, Sayama K, Hashimoto K: Bone morphogenetic protein-2 modulates Wnt and frizzled expression and enhances the canonical pathway of Wnt signaling in normal

keratinocytes. J Dermatol Sci 42:111-9, 2006

Dai X, Sayama K, Yamasaki K, Tohyama M, Shirakata Y, Hanakawa Y, Tokumaru S, Yahata Y, Yang L, Yoshimura A, Koji Hashimoto K: SOCS1 negative feedback of STAT1 activation is a key pathway in the dsRNA-induced innate immune response of human keratinocytes. J Invest Dermatol 126:1574-81, 2006

Yahata Y, Shirakata Y, Tokumaru S, Yang L, Dai X, Tohyama M, Tsuda T, Sayama K, Iwai M, Horiuchi M, Hashimoto K: A novel function of angiotensin II in skin wound healing: Induction of fibroblast and keratinocyte migration by angiotensin II via HB-EGF-mediated EGF receptor transactivation. J Biol Chem 281:13209-16, 2006 (first co-author)

Yang L, Shirakata Y, Shudou M, Dai1X, Tokumaru S, Hirakawa S, Sayama K, Hamuro J, Hashimoto K: New skin-equivalent model from de-epithelialized amnion membrane. Cell Tissue Res 326:69-77, 2006 (first co-author)

Sayama K, Hanakawa Y, Nagai H, Shirakata Y, Dai X, Hirakawa S, Tokumaru S, Tohyama M, Yang L, Sato S, Shizuo A, Hashimoto K: TAK1 is essential for differentiation and the prevention of apoptosis in epidermis. J Biol Chem 281:22013-20, 2006

Ⅳ.　研究成果印刷物（代表論文のみ）

ELSEVIER

EXPERIMENTAL HEMATOLOGY

Experimental Hematology 34 (2006) 642–653

Human herpesvirus 6 impairs differentiation of monocytes to dendritic cells

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(Received 9 September 2005; revised 23 January 2006; accepted 1 February 2006)

Objective. **Monocyte-derived dendritic cells (DCs) play important roles in the immune response against infections and malignancies. Human herpesvirus 6 (HHV-6) infects monocytes and is reactivated in immunodeficient patients. To clarify the mechanisms of HHV-6-induced immunodeficiency, we investigated the effect of HHV-6 infection on differentiation of monocytes to DCs.**

Methods. **Monocytes were inoculated with or without HHV-6 and then allowed to differentiate to myeloid DCs in culture medium containing granulocyte-macrophage colony-stimulating factor and interleukin (IL)-4. The expression of cell surface molecules on DCs and the capacity of the DCs for antigen capture were examined by flow cytometric analysis. Alteration of antigen-presenting capacity induced by HHV-6 infection was examined.**

Results. **The morphology of HHV-6-infected monocyte-derived DCs was distinctly different from that of the DCs derived from mock-infected monocytes. Although expression levels of DC-associated surface antigens, including CD80, CD83, and CD86, were significantly higher on HHV-6-infected monocyte-derived DCs than on DCs derived from mock-infected monocytes, antigen-presenting capacity was significantly lower in the former group. Addition of culture supernatant of HHV-6-infected monocytes resulted in suppression of the T-lymphocyte proliferative response, and anti-IL-10 neutralizing antibody partly inhibited this suppressive effect. The antigen-presenting capacity of DCs generated from a patient with severe HHV-6 reactivation was significantly lower than that of DCs generated from the same patient in the recovery phase.**

Conclusions. **HHV-6 infection induces immunodeficiency via impaired differentiation of DCs. These results present a new concept for the pathogenesis of HHV-6-induced immunodeficiency.** © **2006 International Society for Experimental Hematology. Published by Elsevier Inc.**

Reactivation of human herpesvirus 6 (HHV-6) occurs frequently in patients with immunodeficiency, such as hematopoietic stem cell and organ transplant recipients and those with acquired immune deficiency syndrome (AIDS) [1,2]. This reactivation causes various disorders, including lymphadenitis, pneumonitis, hepatitis, meningoencephalitis, retinitis, infectious mononucleosis-like disease, hemophagocytic syndrome, and drug-induced hypersensitivity syndrome (DIHS) [3–8]. HHV-6 was initially termed human B-lymphotropic virus because of its in vitro tropism for B lymphocytes [9]. However, it is now well known that HHV-6 exhibits tropism mainly for T lymphocytes and monocytes/macrophages and that various kinds of cell, including myeloid precursor cells, megakaryocytes, natural killer cells, fibroblasts, astrocytes, and hepatoma cells, are also susceptible to HHV-6 infection [10–15]. Various immunobiologic alterations of lymphocytes have been observed following infection. HHV-6A infection induces downregulation of CD3, resulting in impairment of T-lymphocyte activation via CD3/T-cell receptor complexes [16,17]. Upregulation of CD4, thus inducing susceptibility to human immunodeficiency virus-1 (HIV-1) infection, has been reported to occur in HHV-6A-infected $CD4^-$ T lymphocytes and natural killer cells [18–20]. HHV-6 infection of T lymphocytes reduces both interleukin (IL)-2 synthesis and the proliferative response to anti-CD3 monoclonal antibody (mAb) and phytohemagglutinin [21]. Transcriptional downregulation of CXCR4 (coreceptor for X4 HIV-1) is induced by HHV-6A and HHV-6B infections [22,23]. In addition to functional

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0301-472X/06 $–see front matter.
doi: 10.1016/j.exphem.2006.02.001

alteration of T lymphocytes, we have reported recently that HHV-6 infection results in impairment of antigen uptake and processing by dendritic cells (DCs) [24].

DCs are professional antigen-presenting cells (APCs) with a remarkable ability to stimulate naive T lymphocytes and generate memory T lymphocytes. Human DCs originating from hematopoietic stem cells can be divided into at least three subpopulations, including interstitial DCs residing in the skin and lymphoid organs and two subsets of blood DCs, the $CD11c^+$ myeloid and $CD11c^-$ plasmacytoid DCs [25]. Because circulating blood DCs are few and are difficult to maintain in culture, monocytes are commonly used as sources of myeloid DCs [26]. There is increasing evidence that maturation of DCs from monocytes may also be relevant in vivo [27,28].

HHV-6 latently infects monocytes [29] and is reactivated in immunodeficient patients. As mentioned above, HHV-6 alters the functions of various immunocompetent cells, and monocyte-derived DCs play important roles in the immune response against infections and malignancies. In light of these facts, clarification of the effects of HHV-6 infection on the differentiation of DCs from monocytes seems an important issue. We therefore investigated the morphologic and immunologic effects of HHV-6 infection on DC differentiation from peripheral blood monocytes.

Materials and methods

Isolation of monocytes and HHV-6 infection

The U1102 strain of HHV-6A and the Z29 strain of HHV-6B were grown in cord blood mononuclear cells that had been stimulated with phytohemagglutinin. Monocytes were isolated from peripheral blood mononuclear cells (PBMCs) of healthy individuals using anti-CD14 mAb-coated magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany). In some experiments, monocytes were isolated from PBMCs of a patient with DIHS, in whom HHV-6 had been severely reactivated, in the acute and recovery phases. The patient's PBMCs were frozen in a liquid nitrogen tank until use. The purity of the monocytes, as determined by their morphology and by flow cytometric analysis using anti-CD14 mAb, was more than 95%. Monocytes were inoculated with HHV-6 at an approximate multiplicity of infection of one 50% tissue culture infective doses.

Differentiation of mock-infected and HHV-6-infected monocytes to DCs

Differentiation of DCs from monocytes was induced, as described previously [30]. Briefly, monocytes were inoculated by incubation with mock stock or HHV-6 stock at 37°C for 2 hours and then washed once. Mock-infected and HHV-6-infected monocytes were then suspended in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 500 U/mL recombinant human IL-4 (Genzyme, Boston, MA, USA), and 800 U/mL recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF; Kirin Brewery, Tokyo, Japan) and cultured in a 5% CO_2 incubator at 37°C. On day 2 or 3 of incubation, half of the medium was exchanged for fresh culture medium supplemented with IL-4 and GM-CSF, and culture was continued. On day 5, the cells were harvested and used as monocyte-derived immature myeloid DCs. Mature myeloid DCs used as the control cells for phenotypic analysis were generated from immature myeloid DCs by adding tumor necrosis factor-α, as described previously [24].

HHV-6 replication in monocyte-derived DCs

Replication of HHV-6 in monocyte-derived DCs was examined by detecting mRNA for the HHV-6 immediate-early and late (*U83*) genes by reverse transcription-polymerase chain reaction (RT-PCR), as described previously [15,31]. Briefly, total RNAs were extracted from HHV-6-inoculated or mock-infected cells, and cDNA was synthesized by reverse transcription with Moloney murine leukemia virus reverse transcriptase. Amplification of the cDNAs by PCR was performed using the following primers: immediate early gene, 5′-TTCTCCAGATGTGCCAGGGAAATCC-3′ and 5′-CATCATTGTTATCGCTTTCACTCTC-3′; late gene, 5′-GTCGACCATGTTCATTTGGCTTTTTATTGTT-3′ and 5′-ATGAATTCTCATGATTCTTTGTCTAATTTC-3′. The expected lengths of the amplified cDNA sequences for the HHV-6 immediate early gene and late gene were 553 bp and 345 bp, respectively. Amplification of cDNA for the β-actin gene was also performed as the control for RT-PCR, using the following primers: 5′-TCCTGTGGCATCCACGAAACT-3′ and 5′-GAAGCATTTGCGGTGGACGAT-3′.

Quantitative real-time PCR for the HHV-6 genome was performed as reported previously [32]. The PCR primers and probe for HHV-6 DNA were selected from the *U31* gene coding a large tegument protein. The upstream primer was 5′-TTTGCAG TCATCACGAT-CGG-3′ and the downstream primer was 5′-AGAGCGACAAATTGGAGGTTTC-3′. A fluorogenic probe, 5′-AGCCACAGCAGCCATCTACATCTGTCAA-3′, was located between the primers. The PCR reaction was performed using a TaqMan PCR kit (PE Applied Biosystems, Foster City, CA, USA), as described previously [32].

Flow cytometric analysis of HHV-6 antigen expression in DCs

Simultaneous detection of surface CD80 and intracellular HHV-6 antigen expression was performed as follows. Mock-infected and HHV-6-infected monocyte-derived DCs were incubated with a fluorescein isothiocyanate (FITC)-conjugated anti-CD80 mAb (Immunotech, Marseilles, France). To further detect intracellular HHV-6 antigen expression, cells were then fixed with 3.0% formaldehyde, permeabilized with 0.05% saponin, and incubated with mAb against the HHV-6 101-kDa virion protein (Chemicon International, Temecula, CA, USA), followed by staining with phycoerythrin-conjugated goat anti-mouse immunoglobulin (Ig) G (Organon Teknika, West Chester, PA, USA). After being washed, the cells were analyzed with a FACSCalibur system (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) equipped with CellQuest software (Becton Dickinson Immunocytometry Systems).

Morphology of mock-infected and HHV-6-infected monocyte-derived DCs

The morphologic differences between mock-infected and HHV-6-infected monocyte-derived DCs were examined under an inverted microscope. Cells fixed on the glass slides by cytospin were stained with May-Giemsa solution. Transmission electron microscopy was performed as described previously [15]. Briefly, the cells were fixed with 2.0% glutaraldehyde in 0.1 M phosphate buffer

(pH 7.4), postfixed with 1% osmium tetroxide, and gradually dehydrated. Samples were embedded in Epon 812, sectioned, stained with uranyl acetate and lead citrate, and examined with a JEM-1230 electron microscope (JEOL, Peabody, MA, USA).

Flow cytometric analysis of cell surface molecule expression

The expression of cell surface molecules on immature and mature DCs differentiated from mock-infected and HHV-6-infected monocytes was examined by flow cytometric analysis using the following mAbs: anti-CD1a (Immunotech), anti-CD40 (PharMingen, San Diego, CA, USA), anti-CD44 (PharMingen), anti-CD80 (Immunotech), anti-CD83 (Immunotech), anti-CD86 (Immunotech), anti-CD209 (DC-SIGN) (Immunotech), anti-HLA class I (PharMingen), and anti-HLA-DR (PharMingen). To block nonspecific binding of mAbs, cells were preincubated with human Ig (Bayer AG, Leverkusen, Germany) at a concentration of 1 mg/mL for 30 minutes before the mAbs were added. After washing, the cells were analyzed as described above.

Flow cytometric analysis of endocytosis

The capacity of the monocyte-derived DCs for antigen capture was examined quantitatively by flow cytometric analysis of endocytosis, as described previously [33]. Briefly, mock-infected and HHV-6-infected monocyte-derived immature and mature DCs were suspended in RPMI 1640 medium supplemented with 10% FCS at 37°C or 4°C. Lucifer yellow CH (Molecular Probes, Eugene, OR, USA) or lysine-fixable FITC-dextran (molecular weight = 40,000; Sigma, St. Louis, MO, USA) was added at a final concentration of 1 mg/mL. The cells were incubated for 30 minutes and washed four times with cold phosphate-buffered saline containing 1% FCS and 0.01% NaN_3 and were analyzed using a FACSCalibur. The staining of cells that had been incubated at 4°C was analyzed as a control.

Presentation of alloantigen to T lymphocytes by monocyte-derived DCs

The allostimulatory capacity of mock-infected and HHV-6-infected monocyte-derived DCs was examined as follows. PBMCs were isolated from donors whose HLA types were nonidentical to those of the monocyte donors. PBMCs (1×10^5) and various numbers of mock-infected and HHV-6-infected monocyte-derived DCs that had been treated with mitomycin C (MMC; Kyowa Hakko, Tokyo, Japan) were cocultured in flat-bottomed microtiter wells each containing 0.2 mL RPMI 1640 medium supplemented with 10% human AB-type serum. The cells were then incubated for 6 days. For the final 16 hours of incubation, 1 μCi [^{3}H]TdR (New England Nuclear, Boston, MA, USA) was added to each well, and the incorporation of [^{3}H]TdR was determined by liquid scintillation counting.

Presentation of exogenous virus antigen to T lymphocytes by monocyte-derived DCs

The exogenous antigen-presentation capacity of mock-infected and HHV-6-infected monocyte-derived DCs was examined using a modification of a previously reported method [34]. Briefly, herpes simplex virus type 1 (HSV-1) antigen was prepared by ultraviolet light irradiation of the virus. PBMCs were isolated from donors who were seropositive for HSV. T lymphocytes were purified from PBMCs by passage through nylon-wool columns and DCs were generated from monocytes from the same donors of T lymphocytes, as described above. T lymphocytes (1×10^5) and various numbers of MMC-treated mock-infected or HHV-6-infected monocyte-derived DCs in 0.2 mL RPMI 1640 medium supplemented with 10% human AB-type serum were seeded in flat-bottomed microtiter wells, to which 0.02 mL virus antigen or control antigen prepared from mock-infected cells was added at the optimal dilution. The cells were cultured for 6 days and the incorporation of [^{3}H]TdR was determined, as described above.

Presentation of peptide antigen to peptide-specific T lymphocytes by monocyte-derived DCs

A chronic myelogenous leukemia-associated fusion protein, the BCR-ABL-specific $CD4^+$ T-lymphocyte clone MY-1, was generated as described previously [35]. MY-1 induces a BCR-ABL fusion peptide (ATGFKQSSKALQRPVAS)-specific and HLA-DRB1*0901-restricted proliferative response. Proliferative response assay of MY-1 was performed using a modification of a previously described method [35]. Briefly, monocytes were isolated from HLA-DRB1*0901-positive donors. MY-1 cells (5×10^4) and various numbers of MMC-treated mock-infected or HHV-6-infected monocyte-derived DCs in 0.2 mL RPMI 1640 medium supplemented with 10% human AB-type serum were seeded into flat-bottomed microtiter wells, to which BCR-ABL peptide was added at 10 μM. The cells were cultured for 3 days and the incorporation of [^{3}H]TdR was determined as described above.

Effect of culture supernatant of mock-infected and HHV-6-infected monocytes on T-cell proliferation

To determine whether soluble suppressor factors of T-lymphocyte activation were produced by HHV-6-infected monocytes, the culture supernatants of mock-infected and HHV-6-infected monocytes were added to MY-1 cells and their proliferation in response to stimulation with BCR-ABL peptide was examined as follows. The culture supernatants of mock-infected and HHV-6-infected monocytes were collected after 3 days of infection and irradiated with ultraviolet to inactivate any HHV-6 produced. The proliferative response of MY-1 to stimulation with BCR-ABL peptide was examined as described above, with a slight modification. Briefly, MY-1 cells (5×10^4) and MMC-treated HLA-DRB1*0901-positive monocyte-derived DCs (2×10^4) in 0.15 mL RPMI 1640 medium supplemented with 10% human AB-type serum and 0.05 mL of culture supernatant of the mock-infected or HHV-6-infected monocytes were seeded into flat-bottomed microtiter wells, to which BCR-ABL peptide was added at 10 μM. The cells were cultured for 3 days and the incorporation of [^{3}H]TdR was determined as described above. In some experiments, the culture supernatants were incubated with anti-IL-10 neutralizing antibody (R&D Systems, Minneapolis, MN, USA) or control antibody at the optimal concentration for 1 hour before being added to the culture wells. In preliminary experiments, more than 90% of the biologic activity of IL-10 was inactivated after incubation with the neutralizing antibody (data not shown).

Results

Replication of HHV-6 in monocyte-derived DCs

Replication of HHV-6 in monocyte-derived DCs was examined by RT-PCR for HHV-6 immediate-early and late genes. RT-PCR analysis clearly showed the presence of