UEA-1、WGA、CD45（白血球系細胞マーカー）抗体を用いて染色した。FACS 解析において M 細胞画分（UEA-1$^+$、WGA$^-$、CD45$^-$）、上皮細胞画分（UEA-1$^-$、WGA$^+$、CD45$^-$）を FACS ソーターによって分離・精製する技術を確立した。

（倫理面への配慮）

本研究における動物実験については国立大学法人東京大学医科学研究所動物実験委員会が定める指針に従って行われた。

C. 研究結果

1）ケモカインによる組織形成プログラム制御：CXCL13 欠損マウスを用いた解析によると胎生期の腸管には CD3$^-$CD4$^+$CD45$^+$細胞の集積を認めず、その結果、パイエル板形成は開始されていなかった。一方 CXCL13 欠損マウスの NALT 原基には CD3$^-$CD4$^+$CD45$^+$細胞の集積が確認された。以上の結果から CXCL13 は NALT 初期形成に関与しないことが示唆された。

2）M 細胞の存在と頻度：組織学的ならびに FACS 解析の結果、正常マウスの NALT 上皮細胞層に M 細胞が存在する事が明らかとなった。さらに GALT の代表組織であるパイエル板の上皮細胞層に M 細胞が存在する事を確認した。さらにその頻度は、パイエル板ドームを形成する上皮細胞層の約 5-8%の頻度で存在している事がわかった。

D. 考察

二次リンパ組織形成に対するケモカインの関与が注目されている。とくに CXCL13 は二次リンパ組織の初期形成や構造維持に必須である。このケモカイン遺伝子欠損マウスを用いて NALT 組織形成における CXCL13 の役割について検討した。その結果 GALT と NALT 間では、CXCL13 が異なる役割をはたしている事が示唆された。つまり GALT の組織形成開始時期には CXCL13 は重要なケモカインであるが、一方で NALT の組織形成開始に関しては必須でないことがわかった。さらに最近の我々の実験から、同ケモカインは同組織においてリンパ節としての微小構造組織形成とその維持には深く関わっているらしい。

M 細胞に関しては NALT を被っている上皮細胞層に UEA$^+$/WGA$^-$の M 細胞が存在する事が確認でき、経鼻ワクチン開発に向けて同細胞が標的となる事を今後明らかにしていく。パウエル板に関しては、M 細胞がドーム中心部から辺縁部に向けて放射状に発達・存在している事がわかった。今後は放射状に存在する M 細胞による抗原取り込み能について検討を進めていく。

E. 結論

本研究で GALT（例　パイエル板）と NALT 組織形成プログラム初期段階におけるリンフォイドケモカイン（例 CXCL13）の両組織間において異なる生物学的役割がある事を示唆出来た。さらに M 細胞については、その存在状態と細胞分離法を確立する事が出来た。

F. 論文発表

1. Kunisawa, J., Kurashima, Y., Gohda. M., Higuchi, M., Ishikawa, I., Ogahara, I., and Kiyono, H. Sphingosine 1-phosphate regulates peritoneal B cell trafficking for subsequent intestinal IgA production. Blood 2007. (in press)

2. Kunisawa, J., Takahashi, I. and Kiyono, H. Intraepithelial lymphocytes: their shared and divergent immunological behaviors in the small and large intestine. Immunol. Rev. 215: 136-153, 2007.

3. Tamagawa, H., Hiroi, T., Mizushima, T., Ito, T., Matsuda, H., and Kiyono, H. Therapeutic effects of roxithromycin in interleukin -10-deficient colitis. Inflamm. Bowel Dis. 2007. (in press)

4. Takayama, N., Igarashi, O., Kweon, MN., and Kiyono, H. Regulatory role of Peyer's patches for the inhibition of OVA-induced allergic diarrhea. Clin. Immunol. 2007. (in press)

5. Kim, N., Knisawa, J., Kweon, MN,, Eog, Ji G., and Kiyono, H. Oral feeding of *Bifidobacterium bifidum* (BGN4) prevents $CD4^+$ CD45RB (high) T cell-mediated inflammatory bowel disease by inhibition of disordered T cell activation. Clin. Immunol. 2007. (in press)

6. Maddaloni, M., Staats, HF., Mierzejewska, D., Hoyt, T., Robonson, A., Callis, G., Kozaki, S., Kiyono, H., McGhee, JR., Fujihashi, K. and Pascual, DW. Mucosal vaccine targeting improves onset of mucosal and systemic immunity to *Botulinum* neurotoxin A. J. Immunol. 177: 5524-5432, 2006.

7. Nochi, T. and Kiyono, H. Innate immunity in the mucosal immune system. Curr Pharm Des. 12: 4203-4213, 2006.

8. Duverger, A., Jackson, RJ., van Ginkel, FW., Fischer, R., Tafaro, A., Leppla, SH., Fujihashi, K., Kiyono, H, McGhee, JR., and Boyaka, PN. *Bacillus anthracis* edema toxin acts as an adjuvant for mucosal immune responses to nasally administered vaccine antigens. J. Immunol. 176: 1776-1783, 2006.

9. Hagiwara, Y., Kawamura, YI., Kataoka, K., Rahima, B., Jackson, RJ., Komase, K., Dohi, T., Boyaka, PN., Takeda, Y., Kiyono, H., McGhee, JR. and Fujihashi, K. A second generation of double mutant cholera toxin adjuvants: enhanced immunity without intracellular trafficking. J. Immunol. 177: 3045-3054, 2006.

10. Jang, MH., Sougawa, N., Tanaka, T., Hirata, T., Hiroi, T., Tohya, K., Guo, Z., Umemoto, E., Ebisuno, Y., Tang, BG., Seoh, JY., Lipp, M., Kiyono, H, and Miyasaka, M. CCR7 is critically important for migration of dendritic cells in intestinal lamina propria to mesenteric lymph nodes. J. Immunol. 176: 803-810, 2006.

11. Fukuyama, S., Nagatake, T., Kim, DY., Takamura, K., Park, EJ., Kaisho, T., Tanaka, N., Kurono, Y. and Kiyono, H. Uniqueness of lymphoid chemokine requirement for the initiation and maturation of NALT organogenesis. J. Immunol. 177: 4276-4280, 2006. (Cutting Edge)

12. 吉田理人、五十嵐脩、寺原和孝、清野宏. 繊毛 M 細胞の形態と機能. 臨床免疫. 45 :236-242, 2006.

13. 廣井隆親、塚越百合子、清野宏 粘膜系好酸球様樹状細胞による免疫寛容制御 感染と免疫 221-230, 2007.

G.学会発表

1. Kiyono, H. 粘膜ワクチン開発へ向けて：新しい展開. The 79th Annual meeting of Japanese Society for Bacteriology. Kanazawa. Japan. March 2006.

2. Kiyono, H. 粘膜免疫を応用した感染症ワクチン開発ストラテジー. The 80th Annual

Meeting of the Japanese Asociation for Infectious Diseases. Tokyo. April 2006.

3. Kurashima, Y., Kunisawa, J., Gohda, M., Higuchi, M., Shimizu, M. and Kiyono H. . Different roles of sphingosine 1-phosphate in the regulation of mobility of mast cells and eosinophilis in peritoneal and intestinal compartments. Immunology 2006, Annual Meeting of The American Association of Immunologists. Massachusetts, USA. May 12th. 2006.

3. Kiyono, H. M cell targeted mucosal vaccine. Immunology 2006, Annual Meeting of The American Association of Immunologists. Massachusetts, USA. May 13th. 2006.

4. Kunisawa, J., Kurashima, Y., Higuchi, M., Gohda, M., Shimizu, M. and Kiyono, H. Sphingosine-1-phosphate-mediated regulatory T cell trafficking in the large intestinal epithelium. Immunology 2006, Annual Meeting of The American Association of Immunologists. Massachusetts, USA. May 13th. 2006.

5. Takayama, N., Igarashi, O., Kweon, MN., and Kiyono, H. Peyer's patch NKT and $CD4^{+}CD25^{+}$ T cell mediated mucosal regulatory network for the control of intestinal allergy. Immunology 2006, Annual Meeting of The American Association of Immunologists. Massachusetts, USA. May 13th. 2006.

6. Gohda, M., Kunisawa, J., Kurashima, Y., Higuchi, M., Shimizu, M., and Kiyono, H. Regulation of peritoneal B cell mobility to intestine and subsequent production of the intestinal IgA by phingosine -1-phosphate Immunology 2006, Annual Meeting of The American Association of Immunologists. Massachusetts, USA. May 14th. 2006.

7. Terahara, K., Igarashi, O., Gotoh, Y., Nochi, T., Yuki, Y., Hiroi, T. and Kiyono, H. Induction of villous M-cells by intestinal environmental factors. Immunology 2006, Annual Meeting of The American Association of Immunologists. Massachusetts, USA. May 14th. 2006.

8. Yuki, Y., Takagi, H., Nochi, T., Yang, L., Hiroi, T., Takaiwa, F. and Kiyono, H. Development of a rice-based oral vaccine. Immunology 2006, Annual Meeting of The American Association of Immunologists. Massachusetts, USA. May 14th. 2006.

9. Terahara, K., Igarashi, O., Yoshida, Y., Nochi, T., Goto, G., Taguchi, F., Beauchemin, N. and Kiyono, H. Novel CEACAM 1 splice variants expressed by murine small intestinal epithelium. The 20th IUBMB International Congress of Biochemistry and Molecular Biology and the 11th FAOBMB Congress. Kyoto, Japan. Jun 17th. 2006.

10. Kiyono, H. Mucosal decision for immunity, tolerance and infection. 2nd the Biomolecule Secretion Research Symposium. Seoul. Korea. October 2006.

11. Kiyono, H. 粘膜免疫：免疫とアレルギーの接点. The 43th Annual meeting of Japanese Society of Pediatric Allergy and Clinical immunology. Chiba.

November 2006.

12. Kiyono, H. Mucosal antigen uptake network : A key for the development of mucosal vaccine. US-Japan Cooperative Medical science Program, 19th Joint Meeting of the AIDS Panels. Kagoshima. December 2006.

13. 國澤純、倉島洋介、樋口森生、合田昌史、清野宏. Sphingosine 1-phosphate receptor differently regulates naïve and activated intraepithelial T lymphocytes. 第36回日本免疫学会総会・学術総会, 大阪市, 大阪府, 12月13日, 2006.

14. 高山尚子、五十嵐脩、権美那、清野宏. Role of NKT cells for the formation of a mucosal regulatory network in the control of intestinal allergy. 第36回日本免疫学会総会・学術総会, 大阪市, 大阪府, 12月13日, 2006.

15. 樋口森生、國澤純、倉島洋介、合田昌史、清野宏. Cytokine regulation of epithelial cell-mediated innate and acquired phases of antigen presentation. 第36回日本免疫学会総会・学術総会, 大阪市, 大阪府, 12月13日, 2006.

16. 野地智法、幸義和、依田昌樹、魚住明央、寺原和孝、金銅瑩、福山聡、五十嵐脩、清野宏. Immunological and biochemical characterization of M-cell by newly established monoclonal antibody. 第36回日本免疫学会総会・学術総会, 大阪市, 大阪府, 12月13日, 2006.

17. 魚住明央、野地智法、依田昌樹、幸義和、五十嵐脩、柴田宏昭、清野宏. Identification and characterization of non-human primate M-cells. 第36回日本免疫学会総会・学術総会, 大阪市, 大阪府, 12月13日, 2006.

H. 知的財産権の出願・登録状況

該当なし

III. 研究成果の刊行に関する一覧表

廣井隆親

1. Hiroi, T. and Takaiwa, F. Peptide immunotherapy for allergic diseases using a rice-based edible vaccine. Curr. Opin. Allergy. Clin. Immunol., 6: 455-460, 2006.

2. Terahara, K., Takahashi, K.G., Nakamura, A., Osada, M., Yoda, M., Hiroi, T., Hirasawa, M., and Mori K. Differences in integrin-dependent phagocytosis among three hemocyte subpopulations of the Pacific oyster "Crassostrea gigas". *Dev. Comp. Immunol.*, 30: 667-683, 2006.

3. Jang, MH., Sougawa, N., Tanaka, T., Hirata, T., Hiroi, T., Tohya, K., Guo, Z., Umemoto, E., Yang, B., Seoh JY., Kiyono, H. and Miyasaka, M. CCR7 is critically important for migration of immunomodulatory dendritic cells in intestinal lamina propria to mesenteric lymph nodes. *J. Immunol.*, 176: 803-810, 2006.

4. Tamagawa, H., Hiroi, T., Mizushima, T., Ito, T., and Kiyono, H. Therapeutic efficacy of roxithromycin on colitis of interleukin-10 deficient mice. *IBD* 2007 (in press).

5. 渡邊伸昌、清野宏、廣井隆親、花粉症対策-米国および日本における現状と将来, *治療学*, 41(1), ライフサイエンス出版, 41: 32-36, 2007.

6. 鈴木一矢、廣井隆親、高岩文雄、清野宏、花粉症緩和米, *Medical Science Digest*, (印刷中)

7. 横山清司、鈴木一矢、高岩文雄、廣井隆親、スギ花粉症緩和米による予防効果、*アレルギーの臨床*、北隆館、 27: 17-23, 2007.

8. 廣井隆親、塚越百合子、清野宏、粘膜系好酸球様樹状細胞による免疫寛容制御 *Annual Review 免疫 2007*. 中外医学社、221-230, 2006.

川口 寧

1. Kato, A., Yamamoto, M., Ohno, T., Tanaka, M., Sata, T., Nishiyama, Y. and Kawaguchi, Y. Herpes Simplex Virus 1-Encoded Protein Kinase UL13 Phosphorylates the Viral Us3 Protein Kinase and Regulates nuclear Localization of Viral Envelopment Factors UL34 and UL31. *J. Virol.*, 80: 1476-1486, 2006.

2. Asai, R., Kato, A., Kato, K., Kanamori-Koyama, M., Sugimoto, K., Sairenji, T., Nishiyama, Y. and Kawaguchi, Y. Epstein-Barr Virus Protein Kinase BGLF4 Is a Virion Tegument Protein That Dissociates From Virions in a Phosphorylation Dependent Process and Phosphorylates the Viral Immediate-Early Protein BZLF1. *J. Virol.*, 80: 5125-5134, 2006.

3. Kudoh, A., Daikoku, T., Ishimi, Y., Kawaguchi, Y., Shirata, N., Iwahori, S., Isomura, H. and Tsurumi, T. Phosphorylation of MCM4 at Sites Inactivating DNA Helicase Activity of the MCM4-6-7 Complex during Epstein-Barr Virus Productive Replication. *J. Virol.*, 80: 10064-10072, 2006.

4. Arii, J., Hushur, O., Kato, K., Kawaguchi, Y., Tohya, Y. and Akashi, H. Construction of an infectious clone of canine herpesvirus genome as a bacterial artificial chromosome. *Microbes and Infection*, 8: 1054-1063, 2006.

5. Koshizuka, T., Kawaguchi, Y., Goshima, F., Mori, I. and Nishiyama, Y. Association of two membrane proteins encoded by herpes simplex virus type2, UL11 and UL56. *Virus Genes*, 32: 157-167, 2006.

高橋秀実

1. Yamanishi S., Iizumi T., Watanabe E., Shimizu M., Kamiya S., Nagata K., Kumagai Y., Fukunaga Y. Takahashi, H. Implications for induction of autoimmunity via activation of B-1 cells by Helicobacter pylori urease. *Infect. Immun.*, 74: 248-256, 2006.

2. Wakabayashi A., Utsuyama M., Hosoda T., Sato K., Takahashi H., Hirokawa K. Induction of immunological tolerance by oral, but not intravenous and intraportal, administration of ovalbumin and the difference between young and old mice. *J. Nutr. Health Aging*, 10: 183-191, 2006.

3. Watanabe Y., Watari E., Matsunaga I., Hiromatsu K., Dascher C.D., Kawashima T., Norose Y., Simizu K., Takahashi, H., Yano I., Sugita M. BCG vaccine elicits both T-cell mediated and humoral immune responses directed against mycobacterial lipid components. *Vaccine*, 24: 5700-5707, 2006.

4. Wakabayashi A, Kumagai Y, Watari E, Shimizu M, Utsuyama M, Hirokawa K, Takahashi H. Importance of gastrointestinal ingestion and macromolecular antigens in the vein for oral tolerance induction. *Immunology*, 119: 167-177, 2006.

5. Nakagawa Y., Kikuchi H., Takahashi H. Molecular analysis of TCR and peptide/MHC interaction using P18-I10-derived peptides with a single D-amino acid substitution. *Biophysical J.*, 2007 (in press).

6. Takahashi M., Watari E., Shinya E., Shimizu T., Takahashi H. Suppression of virus replication via down-modulation of mitochondrial short chain enoyl-CoA hydratase in human glioblastoma cells. *Antiviral Res.*, 2007 (in press).

7. 高橋秀実、癌の免疫療法：丸山ワクチンの作用機序に関する一考察. *日本医科大学医会誌*, 2: 1-2, 2006.

8. 高橋秀実、免疫システムの新たな実態：基本免疫と獲得免疫. *日本感染症学会雑誌*, 80: 463-468, 2006.

9. 新谷英滋、大脇敦子、高橋秀実、DsRed2 を用いたエイズウイルス nef 遺伝子産物と脂質抗原提示分子 CD1a 相互作用の解析. *日本医科大学医会誌*, 2: 134-135, 2006.

10. 高橋秀実、体表面に配置された自然免疫システムと体内を循環する獲得免疫システム. *炎症と免疫*, 14: 449-450, 2006.

11. 高橋秀実、粘膜組織における HIV の拡散と制御. *炎症と免疫*, 14: 479-485, 2006.

12. 飯泉匡、熊谷善博、高橋秀実、Helicobacter pylori 由来 urease の酵素活性を増強させる特異的抗体. 臨床免疫・アレルギー科, 46:205-207, 2006.

13. 新谷英滋、高橋秀実、ヒト免疫不全ウイルス Nef による免疫抑制の機序. 臨床免疫・アレルギー科, 46:222-226, 2006.

14. 高橋秀実、HIV-1 と nef. 炎症と免疫, 14:816-821, 2006.

15. 高橋秀実、持続感染症としての未病. 未病医学入門(日本未病システム学会編), pp. 108-112, 2006.

16. 山西慎吾、神谷茂、高橋秀実、ピロリ菌ウレアーゼによる B-1 細胞活性化作用と自己免疫疾患誘導の可能性. 日本ヘリコバクター学会誌, 2007 (印刷中).

横田恭子

1. Tsunetsugu-Yokota, Y., Ishige, M. and Murakami, M. Attenuated *Salmonella Typhimurimu* expressing codon-opitimized HIV-1 Gag potentiated Gag-specificCD8$^+$ T cell-response in intestinal mucosa. Aids Res. Hum. Retro. 2007. (in press)

2. Yamamoto, T., Isogai, M., Ohtake, K. and Tsunetsugu-Yokota, Y. High and inducible expression of human immunodeficiency virus type 1 (HIV-1) Nef by adenovirus vector does not disturb potent antigen-presentation by monocyte-derived dendritic cells. Microbes Infect., 8: 2522-2530, 2006.

3. Yamamoto, T., Miyoshi, H., Yamamoto, N., Yamamoto, N., Inoue, J-I and Tsunetsugu-Yokota, Y. Lentivirus vectors expressing short hairpin RNAs against the U3-overlapping region of HIV nef inhibit HIV replication and infectivity in primary macrophages. Blood, 108: 3305-3312, 2006.

4. Ishii, K., Hasegawa, H., Nagata, N., Mizutani, T., Morikawa, S., Tashiro, M., Suzuki, T., Taguchi, F., Takemori, T., Tsunetsugu-Yokota, Y. and Miyamura, T. Induction of Protective Immunity against Severe Acute Respiratory Syndrome Coronavirus (SARS- CoV) infection using highly attenuated recombinant- vaccinia Virus DIs. Virology, 351: 368-380, 2006.

5. Tsunetsugu-Yokota, Y., Ohnishi, K., and Takemori, T. Severe acute respiratory syndrome (SARS) coronavirus: application of monoclonal antibodies and development of an effective vaccine. Rev. Med. Virol., 16: 117-131, 2006.

清野 宏

1. Kunisawa, J., Kurashima, Y., Gohda, M., Higuchi, M., Ishikawa, I., Ogahara, I., and Kiyono, H. Sphingosine 1-phosphate regulates peritoneal B cell trafficking for subsequent intestinal IgA production. Blood 2007. (in press)

2. Kunisawa, J., Takahashi, I., and Kiyono, H. Intraepithelial lymphocytes: their shared and divergent immunological behaviors in the small and large intestine. Immunol. Rev., 215: 136-153, 2007.

3. Tamagawa, H., Hiroi, T., Mizushima, T., Ito, T., Matsuda, H., and Kiyono, H. Therapeutic effects of roxithromycin in interleukin -10-deficient colitis. Inflamm. Bowel Dis. 2007. (in press)

4. Takayama, N., Igarashi, O., Kweon, MN., and Kiyono, H. Regulatory role of Peyer's patches for the inhibition of OVA-induced allergic diarrhea. Clin. Immunol., 2007. (in press)

5. Kim, N., Kunisawa, J., Kweon, MN., Eog, Ji G., and Kiyono, H. Oral feeding of *Bifidobacterium bifidum* (BGN4) prevents $CD4^+$ CD45RB (high) T cell-mediated inflammatory bowel disease by inhibition of disordered T cell activation. Clin. Immunol., 2007. (in press)

6. Maddaloni, M., Staats, HF., Mierzejewska, D., Hoyt, T., Robonson, A., Callis, G., Kozaki, S., Kiyono, H., McGhee, JR., Fujihashi, K., and Pascual, DW. Mucosal vaccine targeting improves onset of mucosal and systemic immunity to *Botulinum* neurotoxin A. J. Immunol., 177: 5524-5432, 2006.

7. Nochi, T., and Kiyono, H. Innate immunity in the mucosal immune system. Curr Pharm Des., 12: 4203-4213, 2006.

8. Duverger, A., Jackson, RJ., van Ginkel, FW., Fischer, R., Tafaro, A., Leppla, SH., Fujihashi, K., Kiyono, H., McGhee, JR., and Boyaka, PN. *Bacillus anthracis* edema toxin acts as an adjuvant for mucosal immune

responses to nasally administered vaccine antigens. J. Immunol., 176: 1776-1783, 2006.

9. Hagiwara, Y., Kawamura, YI., Kataoka, K., Rahima, B., Jackson, RJ., Komase, K., Dohi, T., Boyaka, PN., Takeda, Y., Kiyono, H., McGhee, JR. and Fujihashi, K. A second generation of double mutant cholera toxin adjuvants: enhanced immunity without intracellular trafficking. J. Immunol., 177: 3045-3054, 2006.

10. Jang, MH., Sougawa, N., Tanaka, T., Hirata, T., Hiroi, T., Tohya, K., Guo, Z., Umemoto, E., Ebisuno, Y., Tang, BG., Seoh, JY., Lipp, M., Kiyono, H. and Miyasaka, M. CCR7 is critically important for migration of dendritic cells in intestinal lamina propria to mesenteric lymph nodes. J. Immunol., 176: 803-810, 2006.

11. Fukuyama, S., Nagatake, T., Kim, DY., Takamura, K., Park, EJ., Kaisho, T., Tanaka, N., Kurono, Y. and Kiyono H. Uniqueness of lymphoid chemokine requirement for the initiation and maturation of NALT organogenesis. J. Immunol., 177: 4276-4280, 2006. (Cutting Edge)

12. 吉田理人、五十嵐脩、寺原和孝、清野宏、繊毛M細胞の形態と機能. 臨床免疫. 45:236-242, 2006.

13. 廣井隆親、塚越百合子、清野宏、粘膜系好酸球様樹状細胞による免疫寛容制御 感染と免疫 221-230, 2007.

IV. 研究成果の刊行物・別刷

Peptide immunotherapy for allergic diseases using a rice-based edible vaccine

Takachika Hiroi[a] and Fumio Takaiwa[b]

Purpose of review

Plant pollens are the most common cause of seasonal allergic disease. The number of patients undergoing treatment for allergies to the pollen of Japanese cedar (major antigens: Cry j 1 and Cry j 2) has increased steadily each year. Integration of an effective, safe and inexpensive clinical program would be greatly improved by addressing deficiencies in systemically delivered immunotherapy.

Recent findings

We have demonstrated that feeding mice transgenic rice seeds accumulating the T-cell epitope peptides of Cry j 1 and Cry j 2 before systemic challenge with total protein of cedar pollen inhibits the development of allergen-specific IgE, IgG and $CD4^+$ T-cell proliferative responses. The levels of allergen-specific $CD4^+$ T-cell-derived allergy-associated T-helper 2 cytokine of IL-4, IL-5, and IL-13 and histamine release in serum were also significantly decreased. Moreover, clinical symptoms were inhibited in an experimental sneezing-mouse model.

Summary

Plant-based edible vaccine has been shown to be effective for treatment of Japanese cedar pollinosis. When rice seeds containing T-cell epitopes derived from cedar pollen allergens were orally administered to mice, immune tolerance leading to reduction of allergen-specific IgE, T-cell proliferative reaction and histamine could be induced, resulting in suppression of allergic-specific symptoms such as sneezing.

Keywords

immunoglobulin E, oral tolerance, pollen allergy, T-cell epitope, transgenic rice

Curr Opin Allergy Clin Immunol 6:455–460. © 2006 Lippincott Williams & Wilkins.

[a]Department of Allergy and Immunology, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan and [b]Transgenic Crop Research and Develop Center, National Institute of Agrobiological Sciences, Ibaraki, Japan

Correspondence to Dr Fumio Takaiwa, Transgenic Crop Research and Develop Center, National Institute of Agrobiological Sciences, 2-1-2 Kannodai, Tsukuba, Ibaraki 305-8602, Japan
Tel: +81 29 838 8373; fax: +81 29 838 8397; e-mail: takaiwa@nias.affrc.go.jp

Current Opinion in Allergy and Clinical Immunology 2006, 6:455–460

1528-4050

Introduction

Plant pollens are the most common cause of seasonal allergic disease. The number of patients suffering from the pollen of Japanese cedar (*Cryptomeria japonica*) has increased steadily to the point that *Cryptomeria* pollinosis has become a serious social problem in Japan [1]. One epidemiological study reported that about 30% of the general population of Tokyo aged 20–40 years old had circulating IgE antibodies to Japanese cedar pollen [2]. In general, allergen avoidance, pharmacotherapy and immunotherapy are the three main principles of treatment for allergic diseases, and allergen-specific immunotherapy has been used for more than 90 years [3]. Protein extracts from several pollens or dust mites were used to systemically challenge allergic patients, resulting in the reduction of clinical symptoms [4]. Many clinically important allergens have been purified from the extracts of whole proteins [5]. More recently, genes encoding allergens have been cloned for use in immunotherapy. Derivatives of several recombinant allergens, including synthetic peptides (e.g. T-cell-specific epitope peptide), and variants of wild-type recombinant allergens have been employed to avoid the clinical complications of allergic anaphylaxis [6]. In addition to the modification of antigens, mucosal immunization has been notable for the safety and effectiveness of vaccinations as a replacement for systemic inoculation with a hypodermic needle.

In this article, the uniqueness of an edible vaccine that uses multiple T-cell epitopes expressed in rice to induce immune tolerance is reviewed, with an emphasis on future directions for clinical therapeutic potential.

Therapeutic evidence of pollen and mite allergies mediated by antigen-specific immunoglobulin E antibody as type I hypersensitivity

Acute hypersensitivity reactions (type I hypersensitivity) occur through the interaction of allergic antigen with antigen-specific IgE antibody, which is predominantly bound to tissue and mucosal mast cells, and circulating basophiles [7]. This immunological binding via Fc receptors leads to mast cell degranulation and the release of chemical mediators of inflammation [8]. IgE responses are local events occurring at the site of allergen entry at mucosal surfaces and at local lymphoid tissues. IgE production from IgE^+ B cells is induced by antigen-presenting cells and in cooperation with B cells and Th2 cells [9,10].

Of the three main principles of treatment for allergic disease, allergen avoidance has the least possibility for adverse side effects, particularly in children, but can be difficult to implement. For example, removal of all Japanese cedar trees from the urban environment is not a socially acceptable option. For indoor environments, however, control of allergens like dust mites can be more practical. The key to a clinical response is the meticulous elimination of house-dust mite allergens. The effectiveness of a house-dust mite eradication regime has been demonstrated with a greater than 100-fold reduction in room dust-mite content and a concomitant improvement in symptom scores [11].

While helpful, the removal or reduction of allergens is more effective in clinical patient management with pharmacotherapy. Many therapeutic medicines against allergic airway diseases have been developed, such as intranasal corticosteroids and systemic antihistamines. The intranasal corticosteroids significantly improve allergic symptoms [12]. Clinical studies have demonstrated that antihistamine therapies effectively reduce symptoms of airway allergic disease in patients with seasonal allergic rhinitis [13].

Specific immunotherapy for the treatment of allergy patients began in 1911, when it was first investigated by Noon and Freeman [14]. Systemic immunization with protein extracts from several pollens or from dust mites has been demonstrated as an effective therapeutic treatment [4]. The clinical effects obtained through allergen-specific immunotherapy are the result of a decrease in allergen-specific IgE, an increase in blocking IgG, an increase in type 1 T helper (Th1) cytokines, and a reduction in allergen-specific T-cell responsiveness. Specific immunotherapy is associated with a risk of anaphylaxis and, rarely, even death, increasing the need for alternative well tolerated vaccines [15]. Thus, because of its inconvenience, contraindications and risks, the use of allergen-specific immunotherapy by systemic immunization for the management of pollinosis and allergic rhinitis remains a matter for discussion within the medical community. Therefore, there is a critical need to develop a safe and effective treatment for allergic diseases that are active at the mucosal surfaces.

Immunity and tolerance in mucosal immune system

The mucosal immune system of the gastrointestinal tract has several special anatomical and physiological features that help it to carry out its unique functions [16]. One of the mucosal immune systems provides a first line of defense against invading pathogens, including allergic antigens, and creates an appropriate cohabitant situation between the mucosal surfaces of the host and outside environments. Under normal circumstances, the mucosal immune system is tightly regulated with inductive and effector sites, which provide an appropriate immunological homeostasis that is regulated quite differently from systemic immunity [16]. The common mucosal immune system, which interconnects the inductive (e.g. Peyer's patch) and effector (e.g. intestinal lamina propria) tissues for the induction of the antigen-specific IgA response, is well known [16]. The effector sites are an important part of the common mucosal immune system, where actual helper T (Th) cell-regulated antigen-specific IgA synthesis occurs. $CD4^+$ Th cells and their derived cytokines (e.g. IL-5 and IL-6) are essential cellular and molecular elements for the induction of IgA production [17]. On the other hand, the induction of mucosal tolerance can also be provided by mucosal exposure to antigens. Oral administration of large amounts of antigen has been shown to induce systemic unresponsiveness, presumably in the presence of mucosal IgA responses [18]. These immunologically distinct responses in mucosa-associated versus systemic-associated lymphoid tissues were originally termed oral tolerance [18]. Thus, oral tolerance represents the most common responses of the host to the environment.

The development of oral tolerance is an important natural mechanism whereby the host presumably avoids hypersensitivity reactions to ingested food proteins and other antigens. The first report of oral tolerance in 1946 showed that oral administration of the contact-sensitizing compound 2,4-dinitrochlorobenzene did not lead to sensitization, but rather prevented the animal from eliciting an immune response to subsequent intracutaneous injections and cutaneous challenges. Several successful therapeutic trials in animal models of human disease support this view. For example, in experimental autoimmune encephalomyelitis models, preventive doses of myelin basic protein can protect a naïve animal if administered before the onset of disease, probably through anergy or deletion of autoreactive cells [19,20]. Therefore, the development of mucosal tolerance by oral immunization against pollen and mite antigens could be an essential and powerful tool for the inhibition of allergic reactions, including IgE-mediated hypersensitivities such as Type I allergic disease.

The mechanisms in the induction of oral tolerance

For the host to benefit from antigen therapy, it must develop oral tolerance. In general, oral tolerance has been thought to be induced and maintained by Th cells [21]. Initial investigations of oral tolerance determined that systemic tolerance could be induced by administration of two separated doses of antigen. It is now known that oral tolerance in mice occurs after either administration of a single high dose of antigen (10–500 mg) or repeated exposure to lower doses (1–5 mg) [22]. These high-dose

and low-dose tolerances are mediated by distinct mechanisms [22]. Clonal deletion and anergy, which can be elicited by large doses of antigen (high-dose tolerance), are characterized by the absence of antigen-specific T-cell proliferation and decreased IL-2 production, resulting in the establishment of oral tolerance [22]. High-dose oral tolerance-induced deletion occurs by CD95 (FAS)-dependent caspase activation leading to apoptosis [23]. Anergy occurs through T-cell receptor ligation with inadequate costimulation, either by cognate interactions between factors such as CD80 and/or CD86 on antigen-presenting cells with CD28 or cytotoxic T-lymphocyte antigen 4 (CTLA4) on T cells [23]. On the other hand, low-dose tolerance is mediated by the active suppression of immune responses by T cells [22,23]. Low-dose tolerance was regulated by regulatory T cells that can be divided into three subsets, such as $CD4^+CD25^+$ natural regulatory T cells, Th3 cells, and Tr1 cells [22,23]. $CD4^+CD25^+$ regulatory T cells expressed high levels of CTLA4 molecules, as well as the immunosuppressive cytokines IL-10 and TGF-β. TGF-β-producing Th3 cells have also been implicated in low-dose tolerance [22,23]. Thus, the ability to control antigen-specific immunological diseases, including allergic diseases, autoimmunity and inflammation, by oral tolerance is a potentially powerful therapeutic tool.

Oral peptide immunotherapy using T-cell epitopes

While allergen-specific immunotherapy using intact native allergens has been used successfully to treat allergic diseases, this type of therapy has been associated with an increased risk of systemic anaphylaxis mediated by the cross-linking of allergen-specific IgE. To avoid these side effects, peptide immunotherapy using dominant T-cell epitopes has been proposed as a treatment alternative [24,25]. Peptide immunotherapy has been mainly performed by systemic injection, intranasal application or oral administration in animal models. These treatments resulted in a reduction in allergen-specific T-cell proliferation and IgE levels, and in changes in the pattern of cytokine release by Th2 cells. Immune deviation from allergen-specific Th2 to Th1 or Th0 cytokine, or down-regulation of the cytokine response released from both Th1 and Th2 cells have been observed after treatment with the peptide vaccine [26]. The route of intranasal or oral (mucosal) administration of T-cell epitopes has not yet been tested in a clinical trial.

Effective treatment of plant-based edible vaccines for allergic diseases

The feasibility of peptide immunotherapy using a plant-based edible vaccine has been recently examined for treatment of Japanese cedar pollen allergic disease (pollinosis) [27••]. Cry j 1 and Cry j 2 are the major allergens of Japanese cedar pollen, and their predominant T-cell epitopes have been identified for mice [28,29] and humans [30,31]. The nucleotide sequences encoding 14 amino acids of mice Cry j 1 (277–290) and Cry j 2 (246–259) dominant T-cell epitope peptides derived from these allergens were introduced into the C-terminal variable regions of the acidic and basic subunits in the soybean seed-storage protein glycinin A1aB1b cDNA, and expressed in seeds of transgenic rice plants under the control of the rice major seed-storage protein glutelin *GluB-1* promoter [27••]. This fusion protein was specifically expressed in the endosperm of seed and accumulated at a level of 7 μg/grain, or 0.5% of total seed protein. When 10 transgenic rice seeds were fed daily to mice for 4 weeks, the mice showed far less of an immune reaction against the pollen in a systemic challenge than mice fed nontransgenic rice controls, indicating that oral immune tolerance was induced by the rice seed-based vaccine. Treated mice not only had significant reductions in the specific $CD4^+$ T-cell proliferative reaction, but also had reduced allergen-specific IgE and IgG levels. IL-4, IL-5 and IL-13 associated with allergen-specific Th2 cells, and histamine released from mast cells, were also inhibited by feeding with the transgenic rice seeds [27••]. It should be noted that clinical symptoms of allergy (i.e. sneezing) were suppressed when assayed in an experimental sneezing mouse. These results showed that oral immune tolerance was induced by the administration of transgenic rice seeds containing the major T-cell epitopes.

Based on the feasibility of an oral peptide immunotherapy strategy against pollen allergy using rice seed-based vaccines, a human version of the transgenic rice seed-based edible vaccine against Japanese cedar pollinosis has been generated [32•]. An artificial hybrid peptide *7Crp* gene encoding a 96 amino-acid protein of seven linked human dominant T-cell epitope peptides, which are derived from Cry j 1 and Cry j 2, was synthesized using rice seed-optimized codons for each amino acid. The hybrid 7Crp peptide exhibited a positive response in 92% of 48 volunteers with *Criptomeria* pollinosis without binding to their IgE antibodies, indicating that it can be used as a safe and effective tolerogen [31].

In order to enhance expression and accumulation, nucleotide sequences for the N-terminal GluB-1 signal peptide and C-terminal KDER ER (endoplasmic reticulum) retention signal were ligated to *7Crp*. This chimeric gene was inserted into the rice genome via *Agrobacterium*-mediated transformation and specifically expressed in transgenic rice seeds under the control of the glutelin *GluB-1* promoter. The artificial peptide accumulates at a level of about 60 μg/20 mg grain in the edible part (endosperm) of the rice seed. The 7Crp peptide is not accumulated in leaf, stem or embryo tissue despite high mRNA transcript levels, as the 7Crp peptide is deposited only in protein bodies in the rice endosperm. When the

transgenic rice seeds were orally administered to B10.S mice, which recognize only one epitope derived from Cry j 1, both the T-cell proliferative response and IgE levels against Cry j 1 were depressed, thus supporting the efficacy of oral immunotherapy using transgenic rice seeds [32•].

It is interesting to note that the reductions in T-cell proliferative activity are retained even after boiling 7Crp rice for 20 min at 100°C, indicating that oral immune tolerance would be effective with cooked rice. Aside from a slight increase in lysine, the amino acid, lipid, nutritional and mineral composition of 7Crp rice and non-transgenic rice are essentially identical. The small difference in lysine accumulation is likely due to lysine-rich regions in the introduced peptide.

New platform of edible vaccine is for vaccine production

T-cell epitope peptides to be used as tolerogens have been chemically synthesized or produced in recombinant bacteria or yeasts, and then extracted and purified for peptide immunotherapy. This production has to be done in a technical facility and the purification process is very costly. In contrast, peptide vaccines produced in the edible parts of crops make large-scale production efficient, accessible and responsive to demand, because direct delivery is achievable without the need for purification, packaging or partition into effective doses. The production of pharmaceutical proteins in transgenic plants has been limited to less than 1% of total protein in many cases [33,34]. Low expression levels are caused by several factors, including the properties of the allergens themselves.

T-cell epitopes are also good tolerogen candidates because they exhibit no or little binding capacity for the IgE due to the lack of B-cell epitopes and because of conformational changes. When expressed in plant cells, they can be readily accumulated if targeted to the appropriate organelle in a suitable tissue of the desired crop. When edible crops containing parts of an allergen are directly administered without purification, high levels of expression are usually required.

Clinically effective levels of tolerogen peptides for inducing the immune-suppressive response have to be reached for practical use. Low levels of expression can be overcome by increasing promoter activity, and by changing the target tissue, subcellular location, plant species, genetic background, codon usage of the transgene, signal sequences involved in posttranslational control, or others [35]. Many antigens have been expressed in plants as edible vaccines under the control of constitutive promoters and produced in leaves or seeds. When transgenes are expressed in leaves, tubers and roots, transgene products are not highly accumulated in these tissues irrespective of high levels of mRNA transcription. Seed is an ideal plant-production platform because it is a natural storage organ that accumulates storage proteins, starch and lipids, offering ample storage space for foreign recombinant proteins [36]. Seed-specific promoters with high activity have been reported in rice, maize, common bean and wheat [36], and compartment-specific promoters have recently been developed in rice [37]. The GUS reporter gene can be highly expressed in the rice aleurone, sub-aleurone and inner starchy endosperm by the 10 kDa prolamin, GluB glutelin and 26 kDa globulin promoters, respectively [37]. Accumulation levels can also be enhanced by changing the genetic background of a host, such as the low storage protein mutant [38,39]. Furthermore, when expressed in seed, recombinant proteins are remarkably stable without loss of activity for more than a year, even if stored at room temperature.

When a vaccine protein is directly administered through the oral route, antigen accumulated in seed is more resistant than the conventional vaccine to enzymatic digestion in the gastrointestinal tract, suggesting that much smaller amounts of edible vaccine delivered as a seed-storage protein would be effective for inducing oral tolerance. Since the antigens have to be delivered to M cells and intestinal epithelial cells in GALT (gut-associated lymphoid tissue) for the induction of immune tolerance, efficacy at lower concentrations may be due to the concentration of antigen by packaging in the plant cell wall, and further concentrated by compartmentalization in specialized protein body organelles. Furthermore, it may be possible to induce immune tolerance at much lower concentrations if bacterial CTB (cholera toxin B-subunit) or LTB (E. coli heat-labile enterotoxin B-subunit) are used as mucosal delivery carrier molecules, because these proteins are known to facilitate antigen delivery and presentation to the GALT cells.

For commercialization (practical use), there are many hurdles to overcome. There are several risks during the production and delivery stages. The major concern is for the potential contamination of seeds during distribution through the process from the production (harvest) to consumption and out-crossing of pollens with non-transgenic rice close to the field where transgenic rice is cultivated for production of pharmaceuticals. These risks are controllable through regulatory measures.

Conclusion

When a seed-based edible vaccine containing T-cell epitopes derived from Japanese cedar pollen allergens was orally administered, production of IL-4, IL-5 and IL-13 cytokines associated with Th2 was decreased compared with a control group fed nontransgenic rice seeds. Antigen-specific IgE, $CD4^+$ T-cell proliferative

response, and histamine release from the treated mice were also inhibited. Clinical symptoms of allergy (i.e. sneezes) were also alleviated. These results indicate that immune tolerance can be induced by oral administration of seeds containing T-cell epitopes in the form of edible vaccine.

Compared with current competing technologies such as microbial and mammalian cell culture systems, plant expression systems offer several advantages in cost of production, control of production scale, accessibility of the technology, low risk of contamination by human and animal pathogens, and the convenience of stable storage and transportation at room temperature. The human T-cell epitope version of *Cryptomeria* tolerogen edible rice is now available for examination as a safe and effective alternative to traditional immunotherapy, and for environmental and food safety considerations.

Acknowledgements

This work was supported by research grants ('Research for the utilization and industrialization of agricultural biotechnology' and 'Functional analysis of genes relevant to agriculturally important trains in rice genome') from the Ministry of Agriculture, Forestry and Fisheries of Japan to F. Takaiwa, and by Grants-in-Aid from the Ministry of Education, Science Culture and Sports, and a Grant-in-Aid for 2003 Multidisciplinary Research Project from MEXT in Japan to T. Hiroi.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 506–508).

1 Kaneko Y, Motohashi Y, Nakamura H, *et al.* Increasing prevalence of Japanese cedar pollinosis: a meta-regression analysis. Int Arch Allergy Immunol 2005; 136:365–371.

2 Nishihata S, Saito Y. Medical consultation dynamics in Japanese cedar pollinosis patients at an office building clinic in central Tokyo. Nippon Jibiinkoka Gakkai Kaiho 2002; 105:751–758.

3 Hejjaoui A, Dhivert H, Michel FB, *et al.* J. Immunotherapy with a standardized Dermatophagoides pteronyssinus extract. IV. Systemic reactions according to the immunotherapy schedule. J Allergy Clin Immunol 1990; 85:473–479.

4 Walker SM, Varney VA, Gaga M, *et al.* Grass pollen immunotherapy: efficacy and safety during a 4-year follow-up study. Allergy 1995; 50: 405–413.

5 Chapman MD, Platts-Mills TA. Purification and characterization of the major allergen from Dermatophagoides pteronyssinus-antigen P1. J Immunol 1980; 125:587–592.

6 Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. J Allergy Clin Immunol 1998; 102:558–562.

7 Umetsu DT, McIntire JJ, Akbari O, *et al.* Asthma: an epidemic of dysregulated immunity. Nat Immunol 2002; 3:715–720.

8 Reischl IG, Coward WR, Church MK. Molecular consequences of human mast cell activation following immunoglobulin E-high-affinity immunoglobulin E receptor (IgE-FcepsilonRI) interaction. Biochem Pharmacol 1999; 58: 1841–1850.

9 Bellinghausen I, Brand P, Bottcher I, *et al.* Production of interleukin-13 by human dendritic cells after stimulation with protein allergens is a key factor for induction of T helper 2 cytokines and is associated with activation of signal transducer and activator of transcription-6. Immunology 2003; 108:167–176.

10 Dodge IL, Carr MW, Cernadas M, *et al.* IL-6 production by pulmonary dendritic cells impedes Th1 immune responses. J Immunol 2003; 170: 4457–4464.

11 Walshaw MJ, Evans CC. Allergen avoidance in house dust mite sensitive adult asthma. Q J Med 1986; 58:199–215.

12 Foresi A, Pelucchi A, Gherson G, *et al.* Once daily intranasal fluticasone propionate (200 micrograms) reduces nasal symptoms and inflammation but also attenuates the increase in bronchial responsiveness during the pollen season in allergic rhinitis. J Allergy Clin Immunol 1996; 98:274–282.

13 Grant JA, Nicodemus CF, Findlay SR, *et al.* Cetirizine in patients with seasonal rhinitis and concomitant asthma: prospective, randomized, placebo-controlled trial. J Allergy Clin Immunol 1995; 95:923–932.

14 Jager L, Wenz W. Situation and trends of hyposensitisation treatment (author's translation). Allerg Immunol 1979; 25:10–24.

15 Borchers AT, Keen CL, Gershwin ME. Fatalities following allergen immunotherapy. Clin Rev Allergy Immunol 2004; 27:147–158.

16 McGhee JR, Michalek SM, Kiyono H, *et al.* Mucosal immunoregulation: environmental lipopolysaccharide and GALT T lymphocytes regulate the IgA response. Microbiol Immunol 1984; 28:261–280.

17 Xu-Amano J, Beagley KW, Mega J, *et al.* Induction of T helper cells and cytokines for mucosal IgA responses. Adv Exp Med Biol 1992; 327:107–117.

18 Fujihashi K, Kato H, van Ginkel FW, *et al.* A revisit of mucosal IgA immunity and oral tolerance. Acta Odontol Scand 2001; 59:301–308.

19 Jewell SD, Gienapp IE, Cox KL, *et al.* Oral tolerance as therapy for experimental autoimmune encephalomyelitis and multiple sclerosis: demonstration of T cell anergy. Immunol Cell Biol 1998; 76:74–82.

20 Whitacre CC, Song F, Wardrop RM, *et al.* Regulation of autoreactive T cell function by oral tolerance to self-antigens. Ann N Y Acad Sci 2004; 1029:172–179.

21 MacDonald TT. T cell immunity to oral allergens. Curr Opin Immunol 1998; 10:620–627.

22 Weiner HL. Oral tolerance: immune mechanisms and the generation of Th3-type TGF-beta-secreting regulatory cells. Microbes Infect 2001; 3:947–954.

23 Mayer L, Shao L. Therapeutic potential of oral tolerance. Nat Rev Immunol 2004; 4:407–419.

24 Wallner BP, Gefter ML. Peptide therapy for treatment of allergic diseases. Clin Immunol Immunopathol 1996; 80:105–109.

25 Haselden BM, Kay AB, Larche M. Peptide-mediated immune responses in specific immunotherapy. Ant Arch Allergy Immunol 2000; 122:229–237.

26 Larche M, Wraith DC. Peptide-based therapeutic vaccines for allergic and autoimmune diseases. Nature Med 2005; 11:569–576.

27 •• Takagi H, Hiroi T, Yang L, *et al.* A rice-based edible vaccine expressing multiple epitopes induces oral tolerance for inhibition of Th2-mediated IgE responses. Proc Natl Acad Sci USA 2005; 102:17525–17530.
Edible vaccine produced in transgenic rice seed was able to prevent the immune response that triggers allergies. Mice that were fed the vaccine showed a dampened immune reaction to pollen allergen, and allergenic symptom such as sneezing was alleviated compared to those fed nontransgenic rice seed.

28 Hirahara K, Saito S, Serizawa N, *et al.* Oral administration of a dominant T-cell determinant peptide inhibits allergen-specific Th1 and Th2 cell response in Cry j 2-primed mice. J Allergy Clin Immunol 1998; 102:961–967.

29 Yoshitomi T, Hirahara K, Kawaguchi J, *et al.* Three T-cell determinants of Cry j 1 and Cry j 2, the major Japanese cedar pollen antigens, retain their immunogenicity and tolerogenicity in a linked peptide. Immunology 2002; 107:517–522.

30 Sone T, Komiyama N, Shimizu K, *et al.* T cell epitopes in Japanese cedar (Cryptomeria japonica) pollen allergens: choice of major T cell epitopes in Cry j 1 and Cry j 2 toward design of the peptide-based immunotherapeutics for management of Japanese cedar pollinosis. J Immunol 1998; 161:448–457.

31 Hirahara K, Tatsuta T, Takatori T, *et al.* Preclinical evaluation of an immunotherapeutic peptide comprising 7 T-cell determinants of Cry j 1 and Cry j 2, the major Japanese cedar pollen allergens. J Allergy Clin Immunol 2001; 108:94–100.

32 • Takagi H, Saito S, Yang L, *et al.* Oral immunotherapy against a pollen allergy using a seed-based peptide vaccine. Plant Biotech J 2005; 3:521–533.
Authors generated transgenic rice seed which accumulated high concentration of hybrid human T cell epitope peptide derived from cedar pollen allergens.

33 Daniell H, Streatfield SJ, Wycoff K. Medical molecular farming: production of antibodies, biopharmaceuticals and edible vaccines in plants. Trends Plant Sci 2001; 6:219–226.

34 Walmsley AM, Arntzen CJ. Plant cell factories and mucosal vaccines. Curr Opin Biotechnol 2003; 14:145–150.

35 Sala F, Rigano MM, Barbante A, *et al.* Vaccine antigen production in transgenic plants: strategies, gene constructs and perspectives. Vaccine 2003; 21:803–808.

36 Stoger E, Ma JKC, Fischer R, *et al.* Sowing the seeds of success: pharmaceutical proteins from plants. Curr Opin Biotechnol 2005; 16: 167–173.

37 Qu LQ, Takaiwa F. Tissue specific expression and quantitative potential evaluate of seed storage component gene promoters in transgenic rice. Plant Biotech J 2004; 2:113–125.

38 Tada Y, Utsumi S, Takaiwa F. Foreign gene products can be enhanced by introduction into low storage protein mutants. Plant Biotech J 2003; 1:411–422.

39 Twyman RM, Stoger E, Schillberg S, *et al.* Molecular farming in plants: host systems and expression technology. Trends Biotechnol 2003; 21:570–578.

CCR7 Is Critically Important for Migration of Dendritic Cells in Intestinal Lamina Propria to Mesenteric Lymph Nodes[1]

Myoung Ho Jang,[2]* Nagako Sougawa,[2]* Toshiyuki Tanaka,* Takako Hirata,† Takachika Hiroi,‡ Kazuo Tohya,§ Zijin Guo,* Eiji Umemoto,* Yukihiko Ebisuno,* Bo-Gie Yang,* Ju-Young Seoh,¶ Martin Lipp,‖ Hiroshi Kiyono,‡ and Masayuki Miyasaka[3]*

Although dendritic cells (DCs) located in the small intestinal lamina propria (LP-DCs) migrate to mesenteric lymph nodes (MLNs) constitutively, it is unclear which chemokines regulate their trafficking to MLNs. In this study we report that LP-DCs in unperturbed mice require CCR7 to migrate to MLNs. In vitro, LP-DCs expressing CCR7 migrated toward CCL21, although the LP-DCs appeared morphologically and phenotypically immature. In MLNs, DCs bearing the unique LP-DC phenotype ($CD11c^{high}CD8\alpha^{int}CD11b^{low}\alpha_L{}^{low}\beta_7{}^{high}$ and $CD11c^{high}CD8\alpha^{-}CD11b^{high}\alpha_L{}^{low}\beta_7{}^{high}$) were abundant in wild-type mice, but were markedly fewer in CCL19-, CCL21-Ser-deficient *plt/plt* mice and were almost absent in CCR7-deficient mice, indicating the critical importance of CCR7 in LP-DC trafficking to MLNs. Interestingly, $CCR7^+$ DCs in MLNs with the unique LP-DC phenotype had numerous vacuoles containing cellular debris in the cytoplasm, although MLN-DCs themselves were poorly phagocytic, suggesting that the debris was derived from the LP, where the LP-DCs ingested apoptotic intestinal epithelial cells (IECs). Consistent with this, LP-DCs ingested IECs vigorously in vitro. By presenting IEC-associated Ag, the LP-DCs also induce T cells to produce IL-4 and IL-10. Collectively, these results strongly suggest that LP-DCs with unique immunomodulatory activities migrate to MLNs in a CCR7-dependent manner to engage in the presentation of IEC-associated Ags acquired in the LP. *The Journal of Immunology,* **2006, 176: 803–810.**

Dendritic cells (DCs)[4] are cardinal constituents of the immune system and play pivotal roles in the induction of Ag-specific immune responses and the maintenance of self-tolerance (1). DCs are abundant in the small intestine, both in organized lymphoid tissues (Peyer's patches (PPs) and isolated lymphoid follicles (ILFs)) and in the lamina propria (LP), the layer of connective tissue between the epithelium and the muscularis mucosa, where they act as sentinels for incoming Ags. The precise discrimination between harmless Ags and dangerous pathogens by these DCs is a likely key mechanism for the maintenance of gut immune homeostasis (2).

Among the intestinal DCs, the DC subsets in PPs have been characterized in the most detail (3–6). PP-DCs can educate Ag-specific T cells to produce IL-4 and IL-10 (3) and confer gut-homing specificity on T cells (7, 8), indicating that they have unique immune-inductive abilities. In contrast, LP-DCs have been only incompletely characterized, mainly due to difficulty in isolating them. Recent investigations have revealed, however, that LP-DCs of a certain subset extend dendrites in a CX_3CR1-dependent manner to the luminal side of the gut for the uptake of Ags (9, 10). In addition, LP-DCs that reside in the terminal ileum sample commensal bacteria and constitutively express IL-12 p40, indicating that these LP-DCs may be involved in the predisposition to chronic inflammation (11). LP-DCs may also be important in the presentation of bacterial Ags directly to LP B cells (12, 13). LP-DCs obtained from mice treated with Flt3 (FMS-like tyrosine kinase 3) ligand, express high levels of IL-10 and type I IFN and can induce a state of immune hyporesponsiveness upon in vivo transfer (14), suggesting that LP-DCs may have an immunomodulatory role in the gut.

Apart from the LP-DCs, a distinct DC subset has been documented in rat intestinal lymph that can constitutively endocytose apoptotic intestinal epithelial cells (IECs) and transport them to the T cell areas of mesenteric lymph nodes (MLNs). However, it remains unclear whether these DCs are derived from the LP, PPs, and/or other intestinal compartment(s). In addition, their function remains unexplored, although they have been implicated in tolerance induction (15). It has been separately reported that among DCs in the MLNs, the $CD8\alpha^{-}CD11b^{+}$ DC subset plays a critical role in inducing cross-tolerance to food Ags, although it remains to be determined whether these DCs take up dying IECs (16).

DCs are thought to leave peripheral tissues when they receive an inflammatory or danger signal. During this process, DCs begin to mature, and the expression of CCR7 increases (17–19), which allows the DCs to enter lymph vessels and gain access to T cell areas

*Laboratory of Immunodynamics, Department of Microbiology and Immunology, Osaka University Graduate School of Medicine, and †21st Century Center of Excellence Program, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan; ‡Division of Mucosal Immunology, Institute of Medical Science, University of Tokyo, Tokyo, Japan; §Department of Anatomy, Kansai College of Oriental Medicine, Osaka, Japan; ¶Department of Microbiology, College of Medicine, Ewha Woman's University, Seoul, Korea; and ‖Max Delbrück Center for Molecular Medicine, Berlin, Germany

Received for publication September 12, 2005. Accepted for publication October 28, 2005.

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 from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and a grant for Advanced Research on Cancer from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

[2] M.H.J. and N.S. contributed equally to this work.

[3] Address correspondence and reprint requests to Dr. Masayuki Miyasaka, Laboratory of Immunodynamics, Department of Microbiology and Immunology, Osaka University Graduate School of Medicine, C8, 2-2 Yamada-oka, Suita 565-0871, Japan. E-mail address: mmiyasak@orgctl.med.osaka-u.ac.jp

[4] Abbreviations used in this paper: DC, dendritic cell; IEC, intestinal epithelial cell; ILF, isolated lymphoid follicle; LN, lymph node; LP, lamina propria; MLN, mesenteric lymph node; PP, Peyer's patch; SP-DC, splenic DC.

0022-1767/06/$02.00