厚生労働科学研究費補助金（基礎研究成果の臨床応用推進研究事業）
分担研究報告書

高度進行癌に対するペプチドワクチンの臨床研究に関する研究

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研究要旨：HLA-A24およびA2陽性の高度進行肝癌並びに大腸癌患者を対象とし、新規ペプチド32種類を追加してのそれぞれ32種と34種のペプチドを用いて、テーラーメイドペプチドワクチン第1相・早期第2相試験を実施した。即ち、予め末梢血中に存在するペプチド反応性キラーT細胞前駆体をin vitro試験で検索し、高反応性ペプチドから最大4種を選択投与する第I相試験を実施した。有害事象は、いずれもgrade Ⅰ～Ⅱであった。免疫反応性においてもCTL前駆体増強もしくはペプチド特異的IgG抗体発現を過半数の症例で認めた。しかしペプチドワクチン単独による著明な腫瘍縮小効果はなく、延命効果においても延長が認められなかった。但し進行性大腸癌ペプチドワクチンと５－FU系の抗癌剤との併用療法においては単独群に比して優位な生命予後の延長が認められた。

A. 研究目的

HLA拘束性癌特異的キラーT細胞（CTL）株を作製し、それの認識する癌拒絶抗原遺伝子とペプチドを同定し上皮性癌治療用のペプチドワクチン及びペプチド抗体を開発する。また、それらを用いての肝癌、大腸癌を対象とするペプチドワクチン開発の臨床試験を実施する。

B. 研究方法

高度進行・再発肝癌並びに大腸癌を主な対象としてHLA-A24結合性ペプチド３２種、及びHLA-A2結合性ペプチド34種を用いて第Ⅰ相・早期第2相試験を実施した。投与ペプチドの選択は、予め患者末梢血単核球を採取し、in vitroで各ペプチドにより刺激してキラーT前駆体の反応性をIFN-γ産生能で判定し、高反応性ペプチドから最大4種までを選出した。投与ペプチド量は先の臨床試験成績から3.0mg/bodyに統一し、incomplete Freund's adjuvantと混合してemulsion化し、2週間隔で皮下投与した。Primary endpointは、安全性評価であり、NCI-CTCにより人体毒性について検討した。Secondary endpointとしては、免疫反応性について評価した．また、次期第II相試験への移行を考慮し、臨床効果についても併せて検討した。

（倫理面への配慮）

当試験を実施するにあたり、臨床試験実施計画書並びに患者説明文書を作成し、これを当大学の倫理委員会に提出して審査を受け、承諾を得た後に臨床試験を開始した。

C. 研究結果

高度進行・再発肺癌並びに大腸癌患者に対し、テーラーメイド癌ペプチドワクチン療法の第Ⅰ相・早期第2相臨床試験を実施した。その結果、個々の患者において投与されるペプチドに相違を認めた。また、ペプチド投与による有害事象の出現は多岐に亘ったもののいずれもgradeⅡまでと軽微であり、特別重篤な毒性の出現は認めていず、これらペプチドを人体にワクチンとして投与する上での安全性は確認された。一方、ペプチドの反応性をみると、ペプチドの中には全く反応を示さないものや、ある種の癌では多数例で陽性反応を示すものが認められた。またペプチド投与による免疫反応性の増強も高頻度に認め、さらには投与ペプチド特異的なIgG抗体の出現を確認した。一方、臨床効果についてみると、これらの高度進行癌ではペプチド投与のみで明らかな腫瘍縮小効果は得ることができず、生命予後の延長も認められなかった。しかしペプチドワクチン単独投与により、増悪した進行大腸癌症例に対して５－FU系の抗癌剤を併用して症例においては、単独郡に比して優位な生命予後の延長が認められた。

D. 考察

高度進行肝癌や大腸癌症例を対象として施行してきた最大４種類のペプチド（投与量3mg/peptide）を投与するワクチン療法の第I相臨床試験の結果をみると、有害事象としては大半の症例に注射局所の腫脹等を認め、また種々の有害反応の出現もみられたがいずれもGrade Ⅰ～IIで重篤には至らず、primary endpointの安全性は確認された。免疫反応性では、CTL前駆体数の増加は約半数に認められ、さらに新たな所見として患者末梢血中にペプチド特異的IgG抗体の発現を67%に認めた。しかしペプチド単独投与による腫瘍縮小効果は認められず今後の課題となった。これらの結果、高度進行肝癌・大腸癌へのペプ

チドワクチン単独での医薬品化を困難と判断された。一方、5−FU系抗癌剤との併用療法による相乗・相加効果がこれまでの臨床試験の結果より示唆された。次年度以降、非骨髄抑制抗癌剤の種類とその最大投与量設定に関する第一相臨床試験の施行が必要と判断された。

E. 結論

①高度進行肝癌と大腸癌患者を対象として施行してきた最大４種類のペプチド(投与量3mg/peptide)を投与するワクチン療法の第I相臨床試験の安全性は確認された。②ペプチドワクチン投与後の免疫反応性では、細胞性及び液性免疫とも過半数において増強効果が得られた。③ペプチド単独投与による腫瘍縮小効果や生命予後の延長は認められなかった。④しかし、大腸癌では抗癌剤との併用による生命予後延長効果が期待された。

G. 研究発表

1.　論文発表

1-1.　論文発表（英文査読誌掲載論文）

1. Tsuda N, Mochizuki K, Harada M, Sukehiro A, Kawano K, Yamada A, Ushijima K, Sugiyama T, Nishida T, Yamana H, Itoh K, Kamura T : Vaccination with predesignated or evidence-based peptides for patients with recurrent gynecologic cancers. J Immunother 27: 60-72, 2004

2.Noguchi M, Itoh K, Suekane S, Yao A, SuetsuguN, Katagiri K, Yamada A, Yamana H, Noda S: Pha-se I trial of patient-oriented vaccination in HLA-A2-positive patients with metastatic hormone-refractoryprostatic cancer.　Cancer Sci 95:77-84, 2004.

3. Nagamatsu Y, Shima I, Hayashi A, Yamana H, Shirouzu K, Ishitake K: Preoperative spirometry versusexpired gas analysis during exercise testing as predi-ctors of cardiopulmonary complications after lung re-section. Surg Today 34: 107-110, 2004.

4. Mine T, Sato T, Noguchi M, Sasatomi T, Gouha-ra R, Tsuda N, Tanaka S, Shomaru H, Katagiri K, Rikimaru T, Schichijo S, Kamura T,　Hashimoto T, Shirouzu K, Yamata A, Todo S, Itoh K, Yamana H:Humoral responses to peptides correlate with overallsurvival in advanced cancer patients vaccinated with peptide based on pre-existing, peptide specific cellu-lar responses. Clin Cancer Res 10: 929-937, 2004.

5. Sato Y, Maeda Y, Shomura H, Sasatomi T, Takahashi M, Kondo M, Shinohara T, Hida N, Katagiri K,Sato K, Sato M, Yamada A, Yamana H, Harada M, Itoh K, Todo S: A phase I trial of cytotoxic T-lymphocyte precursor-oriented peptide vaccines for colorectal varcinima patients. Br J Cancer 90(7): 1334-1342, 2004. (Apr 5)

6. Tanaka Y, Fujii T, Yamana H, Kato S, Morimatsu M, Shirouzu K: Experimental gene therapy using p21/Waf1 gene for esophageal squamous cell carcinoma by gene gun technology. Int J Mol Med. 14(4):545-551, 2004.(Oct)

7. Kuwano M, Oda Y, Izumi H, Yang SJ, Uchiumi T, Iwamoto Y, Toi M, Fujii T, Yamana H, Kinoshita H, Kamura T, Tsuneyoshi M, Yasumoto K, Kohno K: The role of nuclear Y-box binding protein 1 as a global marker in drug resistance. Mol Cancer Ther 3(11): 1485-1492, 2004.

8. Fujita H, Sueyoshi S, Tanaka T, Matono S, MoriN, Shirouzu K, Yamana H, Suzuki G, Hayabuchi N,Matsui M: Esophagectomy: Is it necessary after chemoradiotherapy for a local advanced T4 esophageal cancer? Prospective randomized trial comparing chemoradiotherapy with surgery versus without sur-gery. World J Surg 9: Epub ahead of print, 2004.

9. Yokoyama G, Fujii T, Tayama K, Yamana H, Kuwano M, Shirouzu K:　PKCδ and MAPK mediateG(1) arrest induced by PMA in SKBR-3 breast ca-ncer cells. Biochem Biophys Res Commun 327: 720-726, 2005.

1-2.　論文発表　(和文査読誌掲載論文)

なし

1-3.　論文発表　(総説・プロシーディング・その他)

1. 唐宇飛、山名秀明：食道癌の集学的治療；とくに癌免疫療法の現状と展望について。消化器外科 27(1)： 99−105, 2004.

2. 峯孝志、由谷茂、伊東恭悟、山名秀明：患者オリエンテッド癌免疫療法。Medical Science Digest 30(4)：129−133, 2004.

3. 藤井輝彦、山名秀明、桑野信彦：第III章　胃癌の治療　8．その他の治療(I)血管阻害薬、臨床消化器内科（増刊号　胃癌治療update 2004) 19(7)： 980−987, 2004.

4. 田中優一、藤田博正、田中寿明、的野吾、森直樹、李美彗、笹原弘子、末吉晋、山名秀明、白水和雄：

食道切除後の再建胃管癌に対し光線力学療法(PDT)および胃部分切除を施行した一例。臨床と研究 81(7):1183-1186, 2004.

5. 山名秀明、安藤暢敏：食道がんに対する術後補助化学療法の有効性。血液・腫瘍科 49(3):311-316, 2004.

6. 唐宇飛、藤井輝彦、田山光介、弥永浩、横山吾郎、山口美樹、堀内彦之、笹冨輝男、高森信三、白水和雄、関直子、山名秀明：抗癌剤・放射線併用免疫細胞療法の効果と患者リンパ球サイトカイン産生に関する検討。癌と化学療法 31(11): 1649-1651, 2004.

7. 堀内彦之、唐宇飛、内田信治、林克実、木下壽文、山名秀明、青柳成明、白水和雄、小金丸雅道：肝転移を伴う進行膵癌の局所癌免疫化学療法の経験。癌と化学療法 31(11): 1752-1754, 2004.

1-4. 論文発表 (著書)

山名秀明：経口摂取不能な末期食道癌患者のQOL向上処置。消化器外科診療二頁の秘訣、北島政樹(編)、ヘルス出版、pp102-103, 2004.

2. 学会発表

2-1. 海外学会発表 (口頭・ポスター発表)

1. Toh U, Mine T, Sasatomi T, Horiuchi H, Fujii T, Ishibashi N, Fujita H, Shirouzu K, Koganemaru M, Yutani S, Yamana H: Intraarterial cell transfer immunotherapy for the patients with refractory liver metastases of recurrent gastrointestinal cancer. 95th Ann-ual Meeting of American Association for Cancer Re-search (2004.3.27-31, Orlando)

2. Yamana H, Toh U: Immunotherapy and cancer. Is it reality? IX World Congress of the International Society for Diseases of the Esophagus (2004.5.27-29,2004, Madrid)

3. Toh U, Yamana H, Fujii T, Sasatomi T, Takamori S, Araki Y, Ogo E, Shirouzu K: Repeated immune cell transfer therapy combined with non myeloablative chemotherapy in patients with refractory recurrent gastrointestinal and lung cancer. 40th Annual Mee-ting of American Society of Clinical Oncology (2004.6.5-8, New Orleans)

4. Lee M, Sueyoshi S, Tanaka T, Tanaka Y, Fujita H, Yamana H, Shirouzu K: A case of penetrated ulcer of the reconstructed gastric tube to the pericardium after Esophagectomy for esophageal cancer. 19th World Congress of International Society for DigestiveSurgery (2004.12.8-11, 2004, Yokohama)

5. Mori N, Fujita H, Sueyoshi S, Tanaka T, Tanaka Y, Yamana H, Shirouzu K: Acid secretion of the gastric tube as an esophageal substitute after radical Esophagectomy for thoracic esophageal cancer. 19th World Congress of International Society for DigestiveSurgery (2004.12.8-11, 2004, Yokohama)

6. Nagano T, Sueyoshi S, Tanaka T, Tanaka Y, Yamana H, Fujita H, Shirouzu K: Clinical Analysis of esophageal cancer associated with other primary malignances. 19th World Congress of International Society for Digestive Surgery (2004.12. 0 8-11, 2004, Yokohama)

7. Sasatomi T, Fukumitsu M, Matono K, Ogata Y,Itoh K, Yamana H, Shirouzu K: Cellular and hormo-nal immune response to recurrent colorectal cancer by peptide vaccination with chemotherapy. 19th Wor-ld Congress of International Society for Digestive Surgery (2004.12.8-11, 2004, Yokohama)

8. Takenaka M, Toh U, Fujii T, Tayama K, Yokoy-ama G, Horiuchi H, Shirouzu K, Kokanemaru M, Yamana H: Intraarterial cell transfer immunotherapy for the patients with refractory liver metastases of recurrent gastrointestinal cancer. 19th World Congress of International Society for Digestive Surgery (2004.12.8-11, 2004, Yokohama)

9. Toh U, Fujii T, Tayama K, Sasatomi T, Shirouzu K, Seki N, Yamana H: The synergistic effects of proteasome inhibitor PS-341 and TRAIL against to human tumor cells. 19th World Congress of Internati-onal Society for Digestive Surgery (2004.12.8-11, 2004, Yokohama)

2-2. 国内学会発表 (口頭・ポスター発表)

1. 藤井輝彦、中村アンナ、横山吾郎、小池健太、三島麻衣、山口美樹、弥永浩、白水和雄、山名秀明、津田英照：ヒト乳癌細胞株SKBR-3におけるantineoplastonの増殖抑制効果の検討。第104回日本外科学会定期学術集会 (2004. 4. 7-9, 大阪)

2. 中村寿、高森信三、藤井輝彦、福永真理、三輪啓介、真栄城兼誉、林明宏、桑野信彦、山名秀明、白水和雄：Gefitinibの肺癌における感受性制御とHerceptin併用に関する抗腫瘍効果の検討。第104回日本外科学会定期学術集会 (2004. 4. 7-9, 大阪)

3. 佐藤裕二、前田好章、正村裕紀、本間重紀、近藤

正男、山名秀明、伊東恭悟、藤堂省：進行・再発消化器癌に対するペプチドワクチン第I/II相試験。第104回日本外科学会定期学術集会（2004.4.7-9，大阪）

4. 末吉晋、田中寿明、笹原弘子、的野吾、森直樹、李美慧、田中優一、藤井輝彦、山名秀明、藤田博正、白水和雄：食道癌リンパ節郭清の限界。 第104回日本外科学会定期学術集会（2004.4.7-9，大阪）

5. 唐宇飛、山名秀明、峯孝志、藤井輝彦、笹冨輝男、堀内彦之、石橋生哉、藤田博正、白水和雄：集学的治療としての細胞免疫化学療法による難治性進行癌の治療。 第104回日本外科学会定期学術集会（2004.4.7-9，大阪）

6. 笹冨輝男、宮城佳昭、鳥越昇二郎、佐藤裕一郎、福光賞真、岸本幸也、的野敬子、金澤昌満、大北亮、石橋生哉、岡洋右、木戸浩一郎、唐宇飛、小篠洋之、荒木靖三、山名秀明、伊東恭悟、緒方裕、白水和雄：進行再発大腸癌に対する免疫化学療法。 第104回日本外科学会定期学術集会（2004.4.7-9，大阪）

7. 前田好章、佐藤裕二、正村裕紀、本間重紀、近藤正男、山名秀明、伊東恭悟、藤堂省：抗腫瘍ペプチドワクチン臨床試験における臨床病理学的因子による予後予測の試み。 第104回日本外科学会定期学術集会（2004.4.7-9，大阪）

8. 森直樹、末吉晋、田中寿明、田中優一、笹原弘子、的野吾、山名秀明、藤田博正、白水和雄：食道癌術後再建胃管の酸分泌能についての検討。 第104回日本外科学会定期学術集会（2004.4.7-9，大阪）

9. 的野吾、田中寿明、末吉晋、笹原弘子、田中優一、森直樹、山名秀明、藤田博正、白水和雄：食道扁平上皮癌におけるgap junctional intercellular communication増加によるHSV-tk/GCV癌遺伝子治療の増強効果。 第104回日本外科学会定期学術集会（2004.4.7-9，大阪）

10. 唐宇飛、藤井輝彦、弥永浩、三島舞衣、横山吾郎、笹冨輝男、石橋生哉、堀内彦之、白水和雄、由谷茂、山名秀明：抗癌剤・放射線併用免疫細胞療法の臨床効果と患者リンパ球サイトカイン産生に関する検討。 第25回日本癌免疫外科研究会、第26回癌局所療法研究会ジョイントミーティング（2004.5.20-21，京都）

11. 田中優一、藤田博正、末吉晋、田中寿明、的野吾、森直樹、笹原弘子、李美慧、山名秀明、白水和雄：化学放射線療法の臨床的効果判定と病理学的効果判定の相関。 第58回日本食道学会（2004.6.24-25，東京）

12. 田中寿明、末吉晋、田中優一、笹原弘子、的野吾、森直樹、山名秀明、白水和雄、藤田博正：食道癌術後再発症例に対するDocetaxel/5-FU/CDDPを用いた化学療法。 第58回日本食道学会（2004.6.24-25，東京）

13. 佐藤史顕、嶋田裕、山名秀明、今村正之：全国食道癌登録データとArtificial Neural Networkを用いた、食道癌の5年予後の予測。 第58回日本食道学会（2004.6.24-25，東京）

14. 森直樹、末吉晋、田中寿明、田中優一、笹原弘子、的野吾、李美慧、山名秀明、藤田博正、白水和雄：アルカリ飲用による腐食性食道狭窄の2例。 第58回日本食道学会（2004.6.24-25，東京）

15. 末吉晋、田中寿明、笹原弘子、的野吾、田中優一、森直樹、李美慧、山名秀明、藤田博正、白水和雄：Open手術からみた食道癌鏡視下手術の評価。第59回日本消化器外科学会定期学術総会（2004.7.21-23，鹿児島）

16. 的野吾、末吉晋、田中寿明、田中優一、笹原弘子、森直樹、李美慧、山名秀明、藤田博正、白水和雄：同時性胸部食道癌・下咽頭癌重複症例の臨床的検討。 第59回日本消化器外科学会定期学術総会（2004.7.21-23，鹿児島）

17. 森直樹、末吉晋、田中寿明、田中優一、笹原弘子、的野吾、李美慧、山名秀明、藤田博正、白水和雄：胸部食道癌術後再建胃管における酸分泌能回復についての検討。 第59回日本消化器外科学会定期学術総会（2004.7.21-23，鹿児島）

18. 田中寿明、末吉晋、田中優一、笹原弘子、的野吾、森直樹、山名秀明、藤田博正、白水和雄：進行食道癌に対するステント治療ー食道・気管ステントー。 第59回日本消化器外科学会定期学術総会（2004.7.21-23，鹿児島）

19. 田中優一、末吉晋、田中寿明、笹原弘子、的野吾、森直樹、李美慧、山名秀明、藤田博正、白水和雄：食道癌術後再建胃管癌、および手術不能胃癌に対する光線力学療法(PDT)。 第59回日本消化器外科学会定期学術総会（2004.7.21-23，鹿児島）

20. 笹冨輝男、的野敬子、大北亮、石橋生哉、唐宇飛、荒木靖三、緒方裕、伊東恭悟、山名秀明、白水和雄：進行再発大腸癌に対する免疫化学併用療法。

第59回日本消化器外科学会定期学術総会（2004.7.21-23，鹿児島）

21. フォトバティ　アバス、藤井輝彦、小野真弓、梁松珠、大家真治、山名秀明、桑野信彦：抗転移遺伝子Cap43の乳癌における発現とホルモン応答。第63回日本癌学会学術総会（2004.9.29-10.1）

22. 野尻久雄、山名秀明：レチノイン酸による顆粒球系終末分化誘導抵抗性HL-60亜株におけるガングリオシド発現能と分化誘導抵抗性との関連性。第63回日本癌学会学術総会（2004.9.29-10.1）

23. 中村寿、藤井輝彦、高森信三、福永真理、小野真弓、山名秀明、白水和雄：非小細胞肺癌に対するZD1839(Irresa)とTrastuzumab(Herceptin)併用に関する増強効果。第63回日本癌学会学術総会（2004.9.29-10.1）

24. 末吉晋、田中寿明、田中優一、笹原弘子、的野吾、森直樹、李美慧、永野剛志、山名秀明、白水和雄、藤田博正：進行胸部食道癌に対する胸筋温存開胸・HALSによる切除再建術。第66回日本臨床外科学会総会（2004.10.13-15，盛岡）

25. 田中寿明、末吉晋、田中優一、笹原弘子、山名秀明、藤田博正、白水和雄：食道癌に対する集学的治療。第66回日本臨床外科学会総会（2004.10.13-15，盛岡）

26. 唐宇飛、山名秀明、藤井輝彦、弥永浩、横山吾郎、笹冨輝男、堀内彦之、石橋生哉、白水和雄：Heterogenetic T細胞免疫療法と非免疫抑制性化学療法との併用による総合的癌免疫療法の展望。第17回日本癌治療学会総会（2004.11.25-26，京都）

27. 笹冨輝男、的野敬子、野副安宏、福光賞貴、山田亮、唐宇飛、石橋生哉、緒方裕、山名秀明、白水和雄：進行再発大腸癌に対する免疫化学併用療法。第17回日本癌治療学会総会（2004.11.25-26，京都）

28. 唐宇飛、藤井輝彦、田山光介、横山吾郎、山口美樹、笹冨輝男、堀内彦之、白水和雄、関直子、山名秀明：ヘテロ細胞免疫療法と非免疫抑制性化学療法との併用及びその臨床的展望。第17回日本バイオセラピィ学会学術集会総会（2004.11.25-26，北九州）

29. 笹冨輝男、唐宇飛、荒木靖三、関直子、緒方裕、山名秀明、白水和雄：癌活性化リンパ球とプロテアソーム阻害薬との併用による抗腫瘍効果。第17回日本バイオセラピィ学会学術集会総会（2004.11.25-26，北九州）

H. 知的財産権の出願・登録状況（予定を含む。）

1. 特許取得
   特になし

2. 実用新案登録
   特になし

3. その他
   特になし

厚生労働科学研究費補助金（基礎研究成果の臨床応用推進研究事業）
分担研究報告書

悪性神経膠腫に対するペプチドワクチンの臨床研究に関する研究

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研究要旨：HLA-A2またはA24陽性の再発、再燃胃癌・悪性神経膠腫に対するテーラーメイドペプチドワクチンの第Ⅰ相、第Ⅱ相試験にてこれまでにGrade III glioma 7例及びGrade IV 18例を経験した。投与部炎症反応が主体でありgrade3以上の有害事象は皆無であった。免疫誘導能は細胞性及び液性免疫の両者において66%以上と高頻度に認められた。臨床効果は。5 PR, 8 SD, 8PDであった。以上よりテーラーメイド型ペプチドワクチンの安全性、免疫反応および臨床効果が実証された。

A. 研究目的

悪性神経膠腫の再発例の予後は惨憺たるもので、化学療法や放射線療法の進歩に加え、最近のモノクローナル抗体、シグナル伝達に関わる薬剤などの進歩により生存率の延長が見られるが、そのほとんどの症例は半年以内に死亡する。その原因は、抗癌剤抵抗性の獲得や耐性、患者の全身状態の低下により必要量が投与できない等の理由である。また、それらの治療に対する無効例ではQOLを低下させる。従って、これらの腫瘍に新しい方法論が必要である。　今回悪性神経膠腫の再発例に対してpeptide vaccineの有効性を検討した。

B. 研究方法

テーラーメイド癌ペプチドワクチン第I相・第II相臨床試験を悪性神経膠腫症例に実施した。即ち、HLA-A24陽性及びA2陽性の癌患者を対象として開発された３０種類（A24用14種類、A2用16種類）の癌抗原由来ペプチドのうち、患者の特異免疫系（末梢血リンパ球中のＴ細胞）に高頻度に認識されることが先行する臨床試験にて確認されたペプチドを最大4種類投与した。安全性・特異的免疫誘導能、及び臨床効果と投与前キラーＴ細胞との相関を解析した。

（倫理面への配慮）

1)本研究は「ヒトのクローンに関する研究等」に該当するおそれは無いと判断される。2) in vitroにて癌特異的キラーT細胞を誘導することを目的として採血する場合は、本研究分担者、及び、他の研究協力医が直接患者に十分時間をかけその目的を説明し、理解と同意が得られた場合に限って15～30ml採血して研究に使用している。3) 第Ⅰ相臨床試験についての配慮点：GMPグレードのペプチドを米国NCI外科における臨床試験の基準にのっとり米国MPS社へ依頼して作製し(Nature Med., 4: 321, 1998) 無菌試験及びシークエンスを本研究室にて再確認した後、本学内での審査委員会での審査を経て臨床試験を

実施している。4) 臨床試験実施においては、本研究の分担者（医師）が専任の臨床研究看護士とともに被験者から文書での自由意志による十分な説明を受けた上での同意（インフォームド・コンセント）を得て実施している。さらに、担当医師や看護士を中心として被験者及びその家族の疑問に答え不安に対応するためのカウンセリングを行う体制をとっている。

C. 研究結果

HLA-A2またはA24陽性の悪性神経膠腫に対するテーラーメイドペプチドワクチンの第Ⅰ相、第Ⅱ相試験の有害事象は、経験した２５症例において、投与部炎症反応が主体でありgrade3以上の有害事象は皆無であった。免疫誘導能は細胞性及び液性免疫の両者において66%以上と高頻度に認められた。臨床効果は。5 PR, 8SD, 8PDであった。テーラーメイドペプチドワクチンの安全性と腫瘍特異的免疫誘導能を確認された。

D. 考察

HLA-A24およびA02の悪性神経膠腫を対象としたテーラーメイド癌ペプチドワクチン第I/II相試験は毒性を認めず終了し、本療法は安全に施行できると考えられた。臨床効果に関して単独でのＰＲ5症例が得られ、ワクチン単独療法での優位な臨床効果が得られ、医薬品化が可能と判断された。

特筆すべき点は。投与前血液中のペプチド特異キラーＴ細胞数と強い相関が認められたことであり、

テーラーメイド型投与の優位性が立証されたと判断される。

E. 結論

HLA-A2またはA24陽性の悪性神経膠腫に対するテーラーメイドペプチドワクチンの第Ⅰ相、第Ⅱ相試験の安全性を確認した。また、高い免疫誘導能（細胞性及び液性免疫）も確認した。臨床効果は２５例中5 PR, ８SD, 8PDであった。

G. 研究発表

1. 論文発表

1-1. 論文発表 （英文査読誌掲載論文）

1. Yamanaka R, Xanthopoulos KG.: Development of improved Sindbis virus-based DNA expression vector. DNA Cell Biol 23(2):75-80, 2004

2. Yamanaka R: Alpha Virus Vectors for Cancer Gene Therapy (Review). Int J Oncol. Apr;24(4):919-923,2004.

3. Yamanaka R,Tanaka R: Advances for the treatment of primary central nervous system lymphoma (Review). Oncol Rep 12:563-568, 2004.

4. Yamanaka R, Tanaka R : Gene Therapy ofBrain Tumor with Endostatin. Drug of Today 40(11):931-934,2004

1-2. 論文発表 （和文査読誌掲載論文）

なし

1-3. 論文発表 （総説・プロシーディング・その他）

1. Yamanaka R, Homma J, Yajima N, Tsuchya N, Tanaka R Vaccination of recurrent glioma patients with tumour lysate-pulsed dendritic cells elicits immune responses: results of a clinical phase I/II trial Molecular Ther 9 (1), S98, 2004

2. Yamanaka R, Homma J, Yajima N, Tsuchya N, Takahashi M, Tanaka R Vaccination of recurrent glioma patients with tumour lysate-pulsed dendritic cells elicits immune responses: results of a clinical phase I/II trial Proc. Am. Assoc. Cancer Res. 45, 1237, 2004

3. Homma J, Yamanaka R, Abe T, Narita M, Takahashi M, , Tanaka R Vaccination of recurrent glioma patients with tumour lysate-pulsed dendritic cells elicits immune responses: results of a clinical phase I/II trial Abstract book for the 3rd Meeting of the AsianSociety for Neuro-Oncology, pp7, 2004

4. Yamanaka R, Yajima N, Yamada A, Itoh, K, Mine T, Tanaka R Immunological evaluation of personalized peptide vaccination for patients with malignant glioma: the results of a clinical phase I trialAbstract book for the 3rd Meeting of the Asian Society for Neuro-Oncology, pp7-8, 2004

1-4. 論文発表 （著書）

なし

2. 学会発表

2-1. 海外学会発表（口頭・ポスター発表）

1. Yamanaka R, Homma J, Abe T, Yajima N , Tsuchiya N, Kobayashi T , Narita M , Takahashi M , Tanaka R Vaccination of recurrent glioma patients with tumour lysate-pulsed dendritic cells elicits immune responses: results of a clinical phase I/II trial、The American Association of Gene Therapy's 7th Annual Meeting, Minneapolis, MN, USA June 2-6,2004

2. Homma J, Yamanaka R, Abe T, Narita M, Takahashi M, , Tanaka R Vaccination of recurrent glioma patients with tumour lysate-pulsed dendritic cells elicits immune responses: results of a clinical phase I/II trial. The 3rd Meeting of the Asian Society for Neuro-Oncology, Shanghai, November 19-22, 2004

3. Yamanaka R, Yajima N, Yamada A, Itoh, K, Mine T, Tanaka R Immunological evaluation of personalized peptide vaccination for patients with malignant glioma: the results of a clinical phase I trial. The 3rd Meeting of the Asian Society for Neuro-Oncology, Shanghai, November 19-22, 2004

2-2. 国内学会発表（口頭・ポスター発表）

1. 山中龍也 中枢神経系原発悪性リンパ腫の当科における治療成績、ミニシンポジウム「節外性リンパ腫の治療」、新潟リンフォーマフォーラム2004、新潟市、3月19日、2004

2. 山中龍也、森井研、高橋英明、恩田清、田中隆一 ProMace MOPP Hybrid 新潟大学変法による脳原発悪性リンパ腫の治療成績、第28回北日本脳神経外科連合会学術集会、福井市、5月6日－7日、2004

3. 山中龍也、芥川茂、田口文子、矢島直樹、土屋尚人、森井 研、高橋英明、田中隆一、西條長宏、西尾和人悪性脳腫瘍症例の治療感受性を規定する遺伝子発現プロファイル解析、第22回日本脳腫瘍病

理学会、新潟市、5月20日－21日、2004

4. 矢島直樹、山中龍也、土屋尚人、本間順平、山田亮、伊東恭悟、田中隆一 悪性脳腫瘍患者における腫瘍抗原SART3, Lck, MRP3の発現, 第22回日本脳腫瘍病理学会、新潟市、5月20日－21日、2004

5. 峯孝志、矢島直樹、山中龍也、土屋尚人、本間順平、山田亮、伊東恭悟、田中隆一 悪性神経膠腫に対するテーラーメイド癌ペプチドワクチン療法第I/II相臨床試験、第8回基盤的癌免疫研究会総会、札幌、7月15日-7月16日、2004

6. 山中龍也、本間順平、矢島直樹、土屋尚人、阿部崇、成田美和子、高橋益広、田中隆一
再発悪性神経膠腫に対する樹状細胞を用いた腫瘍特異的免疫療法の第I/II相臨床試験、第8回基盤的癌免疫研究会総会、札幌、7月15日-7月16日、2004

7. 矢島直樹、山中龍也、土屋尚人、本間順平、峯孝志、山田亮、伊東恭悟、田中隆一 悪性神経膠腫に対するCTL precursor-orientedペプチドワクチン療法第I /II相臨床試験, 第5回分子脳神経外科学会、東京大学医学部鉄門記念講堂, 9月4日-9月5日、2004

8. 本間順平、山中龍也、矢島直樹、土屋尚人、阿部崇、成田美和子、高橋益広、田中隆一
再発悪性神経膠腫に対する樹状細胞を用いた腫瘍特異的免疫療法の第I/II相臨床試験、第5回分子脳神経外科学会、東京大学医学部鉄門記念講堂, 9月4日-9月5日、2004

9. 山中龍也、矢島直樹、土屋尚人、本間順平、田中隆一、 三木徹 Guanine Nucleotide Exchange factor OSTへの結合分子の解析、第5回分子脳神経外科学会、東京大学医学部鉄門記念講堂, 9月4日-9月5日、2004

10. 矢島直樹、山中龍也、土屋尚人、本間順平、峯孝志、山田亮、伊東恭悟、田中隆一 悪性神経膠腫に対するCTL precursor-orientedペプチドワクチン療法第I /II相臨床試験, 第63回日本癌学会総会、福岡、9月29日－10月1日、2004

11. 本間順平、山中龍也、矢島直樹、土屋尚人、阿部崇、成田美和子、高橋益広、田中隆一
再発悪性神経膠腫に対する樹状細胞を用いた腫瘍特異的免疫療法の第I/II相臨床試験、第63回日本癌学会総会、福岡、9月29日－10月1日、2004

12. 矢島直樹、山中龍也、土屋尚人、本間順平、峯孝志、山田亮、伊東恭悟、田中隆一 悪性神経膠腫に対するCTL precursor-orientedペプチドワクチン療法第I /II相臨床試験, 第63回日本脳神経外科学会総会、名古屋、10月6日－8日、2004

13. 本間順平、山中龍也、矢島直樹、土屋尚人、阿部崇、成田美和子、高橋益広、田中隆一 再発悪性神経膠腫に対する樹状細胞を用いた腫瘍特異的免疫療法の第I/II相臨床試験、第63回日本脳神経外科学会総会、名古屋、10月6日－8日、2004

14. 山中龍也、矢島直樹、土屋尚人、本間順平、峯孝志、山田亮、伊東恭悟、田中隆一 悪性神経膠腫に対するテーラーメイドペプチドワクチン療法第I /II相臨床試験, 第63回日本脳神経外科学会総会、名古屋、10月6日－8日、2004

15. 土屋尚人、山中龍也、矢島直樹、田中隆一、池中一裕 HPLC(高速液体クロマトグラフィー)を用いたGlioma組織内N-結合型糖タンパク質糖鎖の解析、第63回日本脳神経外科学会総会、名古屋、10月6日－8日、2004

16. 矢島直樹、山中龍也、土屋尚人、本間順平、峯孝志、山田亮、伊東恭悟、田中隆一 悪性神経膠腫に対するCTL precursor-orientedペプチドワクチン療法第I /II相臨床試験, 第22回日本脳腫瘍学会、松島、10月17日－19日、2004

17. 本間順平、山中龍也、矢島直樹、土屋尚人、阿部崇、成田美和子、高橋益広、田中隆一
再発悪性神経膠腫に対する樹状細胞を用いた腫瘍特異的免疫療法の第I/II相臨床試験、第22回日本脳腫瘍学会、松島、10月17日－19日、2004

18. 山中龍也、矢島直樹、土屋尚人、本間順平、峯孝志、山田亮、伊東恭悟、田中隆一 悪性神経膠腫に対するテーラーメイドペプチドワクチン療法第I /II相臨床試験-治験に向けて, 第22回日本脳腫瘍学会、松島、10月17日－19日、2004

19. 山中龍也、矢島直樹、土屋尚人、本間順平、遠藤俊郎、峯孝志、山田亮、伊東恭悟、田中隆一 中枢神経系悪性黒色腫に対するCTL precursor-orientedペプチドワクチン療法第I /II相臨床試験, 第22回日本脳腫瘍学会、松島、10月17日－19日、2004

20. Yamanaka R, Phase I/II clinical trial of CTL precursor-oriented personalized peptide vaccine therapy for patients with malignant glioma. 21世紀のCOEプログラム「先端的な癌治療研究の拠点」国際シンポジウ

ム, 久留米大学、 11月27日、2004

H. 知的財産権の出願・登録状況(予定を含む。)

1. 特許取得
(出願番号) 特願2004-247849、
(発明者) 土屋尚人、山中龍也、池中一裕、
(発明の名称) 脳腫瘍の検出方法及びそれに用いる脳腫瘍の検出物質

2. 実用新案登録
特になし

3. その他
特になし

研究成果の刊行に関する一覧表

英文査読誌　(17編抜粋)

1. Matsueda S., Kobayashi K., Nonaka Y., Noguchi M., Itoh K., Harada M.: Identification of new prostate stem cell antigen-derived peptides immunogenic in HLA-A2+ patients with hormone-refractory prostate cancer. Cancer Immunol. Immunother 53, 479-489, 2004.

2. Sato Y., Maeda Y., Shomura H., Sasatomi T., Takahashi M., Une Y., Kondo M., Shinohara T., Hida N., Katagiri K., Sato K., Sato M., Yamada A., Yamana H., Harada M., Itoh K., Todo S.: A phase I trial of cytotoxic T-lymphocyte precursor-oriented peptide vaccines for colorectal carcinoma patients Br. J. Cancer 90, 1334-1342, 2004.

3. Shomura, H., Shichijo, S., Matsueda, S., Kawakami, T., Sato, Y., Todo, S., and Itoh, K. Identification of epidermal growth factor receptor-derived peptides immunogenic for HLA-A2+ cancer patients. Br. J. Cancer 90, 1563-1571, 2004

4. Noguchi M., Itoh K., Suekane, S., Morinaga A., Sukehiro A., Suetsugu N., Katagiri K., Yamada A., Noda S. :Immunological monitoring during combination of patient-oriented peptide vaccination and estramustine phosphate in patients with metastatic hormone refractory prostate cancer. Prostate 60,32-45, 2004.

5. Matsueda, S., Yao, A., Ishihara, Y., Ogata, R., Noguchi, M., Itoh, K., and Harada M. A prostate stem cell antigen-derived peptide immunogenic in HLA-A24+ prostate cancer patients. Prostate, 60, 205-213, 2004

6. Azuma K., Shichijo, S., Shomura H., Matsueda S., Fujii T., Itoh K.: Identification of HER2/neu-derived peptides capable of inducing both cellular and humoral immune responses in HLA-A24 positive breast cancer patients. Breast Cancer Res. Treat. 86, 19-29, 2004

7. Yao A., Harada M., Matsueda S., Ishihara Y., Shomura H., Noguchi M., Matsuoka K., Hara I., Kamidono S., Itoh K.: Identification of parathyroid hormone-related protein-derived peptides immunogenic in human histocompatibility leukocyte antigen-A24+ prostate cancer patients. Br J Cancer 91, 287-296, 2004.

8. Sasada T., Takedatsu H., Azuma K., Koga M., Maeda Y.,Shichijo S., Shoumura H., Hirai T., Takabayashi A., Itoh K.: Immediate early response gene X-1, a stress -inducible antiapoptotic gene, encodes cytotoxic T-lymphocyte(CTL) epitopes capable of inducing human leukocyte antigen-A33-restricted and tumor-reactive CTLs in gastric cancer patients. Cancer Res 64, 2882-2888, 2004

9. Mochizuki K., Sato Y., Tsuda N., Shomura H., Sakamoto M., Matsuura K., Ushijima K., Maeda Y., Katagiri K., Yamada A., Todo S., Kamura T., Harada M., Itoh K.: Immunological evaluation of vaccination with pre-designated peptides frequently selected as vaccine candidates in an individualized peptide vaccination regimen. Int J Oncol 25, 121-131, 2004.

10. Shomura H., Shichijo S., Komatsu N., Matsueda S., Mine T., Rikimaru T., Sato Y., Todo S., Itoh K.: Identification of epidermal growth factor receptor-derived peptides recognized by both cellular and humoral immune responses in HLA-A24+ non-small cell lung cancer patients. Eur J Cancer 40, 1776-1786, 2004.

11. Takedatsu H., Okamura T., Yoshimoto K., Harada M., Koga M., Shichijo S., Sata M., Itoh K.: Expression of epithelial cancer-related antigens in hematologic malignancies applicable for peptide-based immunotherapy. J Immunother 27, 289-297, 2004.

12. Ogata R., Matsueda S., Yao A., Noguchi M., Itoh K., Harada M.: Identification of polycomb group protein enhancer of zeste homolog 2(EZH2)-derived peptides immunogenic in HLA-A24+ prostate cancer patients. Prostate 60, 273-281, 2004.

13. Shichijo S., Azuma K., Komatsu N., Ito M., MaedaY., Ishihara Y., Itoh K.: Two proliferation-related proteins, TYMS and PGK1, could be new cytotoxic T lymphocyte-directed tumor-associated antigens of HLA-A2+ colon cancer. Clin Cancer Res 10, 5828-5836, 2004.

14. Azuma K, Sasada T, Takedatsu H, Shomura H, Koga M, Maeda Y, Yao A, Hirai T, Takabayashi A, Shichijo S, and Itoh K. *Ran*, a small GTPase gene, encodes CTL epitopes capable of inducing HLA-A33-restricted and tumor-reactive CTLs in cancer patients. Clin. Cancer Res. 10, 6695-6702, 2004

15. Noguchi M., Itoh K., Yao A., Mine T., Yamada A., Obata Y., Furuta M., Harada M., Siekane S., Matsuoka K.: Immunological evaluation of individualized peptide vaccination with a low dose of estramustine for HLA-A24+ HRPC patients. Prostate (in press), 2004.

16. Sato Y., Kondo M., Kohashi S., Takahashi N., Takahashi S., Sinohara T., Ono K., Matsuda M., Ryoma Y., Shiroto H., Kondo Y., Uchino J., Saito K., and Todo S. : A randomized controlled study of immunochemotherapy with OK-432 after curative surgery for gastric cancer. J Immunother. 27, 394-397, 2004.

17. Yamanaka R., Xanthopoulos KG.: Development of improved sindbis virus-based DNA expression vector. DNA Cell Biol 23(2):75-80, 2004.

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ORIGINAL ARTICLE

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Identification of new prostate stem cell antigen-derived peptides immunogenic in HLA-A2$^+$ patients with hormone-refractory prostate cancer

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Abstract *Purpose* Prostate cancer refractory to hormonal manipulation requires new treatment modalities. In the present study we attempted to identify prostate stem cell antigen (PSCA)-derived peptides immunogenic in HLA-A2$^+$ prostate cancer patients in order to develop peptide-based immunotherapy against hormone-refractory prostate cancer (HRPC).
Methods Eleven different PSCA-derived peptides, which were prepared based on the HLA-A2 binding motif, were examined to determine whether they would be recognized by cellular and humoral immune responses in 12 HLA-A2$^+$ patients (11 with HRPC and 1 with non-HRPC).
Results Among the PSCA-derived peptides, PSCA 7–15 and PSCA 21–30 peptides effectively induced HLA-A2-restricted peptide-specific and tumor-reactive cytotoxic T lymphocytes (CTLs) from peripheral blood mononuclear cells (PBMCs) of HLA-A2$^+$ patients. The PSCA 21–30 peptide was capable of inducing peptide-specific CTLs in both cancer patients and healthy donors, whereas the PSCA 7–15 peptide was immunogenic in only cancer patients. Immunoglobulin G (IgG) reactive to the PSCA 21–30 peptide was detected in plasma of most patients and healthy donors, whereas IgG reactive to PSCA 7–15 was undetectable in all cases. These results indicate that the former peptide elicits both cellular and humoral immune responses in both patients and healthy donors, whereas the latter elicits only cellular responses in patients.
Conclusion These two PSCA peptides should be considered for use in clinical trials of immunotherapy for HLA-A2$^+$ HRPC patients.

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Keywords Prostate cancer · PSCA · CTLs · Peptide · Antibody

Introduction

Prostate cancer is one of the most common cancers in older men [5]. Although hormone ablation therapy can temporarily palliate patients with advanced disease, the progression to hormone-refractory prostate cancer (HRPC) is inevitable in most cases [17]; therefore, the development of novel therapeutic modalities for the treatment of HRPC is necessary. One such therapy could be peptide-based immunotherapy, as recent advances in tumor immunology have enabled us to identify many genes encoding tumor antigens and their peptides that are recognized by cytotoxic T lymphocytes (CTLs) [1, 28]. Several prostate-specific antigens and antigen-derived peptides have also been reported, including prostate-specific antigen (PSA) [2, 3, 31] prostate-specific membrane antigen (PSMA) [10, 30], and prostatic acid phosphatase (PAP) [12, 25]. Some of these antigen-derived peptides have been used in the treatment of prostate cancer patients, but the clinical responses observed thus far have been unsatisfactory [19, 20, 21]; therefore, new antigens and peptides suitable for use in specific immunotherapy for patients with HRPC are needed.

The prostate stem cell antigen (PSCA) is a recently identified antigen expressed on the cell surface of prostate cancer cells [27]. This antigen is a glycosylphoshatidylinositol-anchored protein, and is overexpressed by both androgen-dependent and androgen-independent prostate cancers [27], suggesting that this antigen could be a good candidate for specific immunotherapy for patients with HRPC. In addition, immunotherapy targeting on PSCA could be promising for the treatment of patients with bone metastases, as the expression of PSCA in prostate cancer is higher in metastases than in primary tumors [6].

We report in this study new PSCA-derived peptides that can be recognized by both cellular and humoral immune responses in HLA-A2$^+$ prostate cancer patients.

Materials and methods

Patients

Twelve HLA-A2$^+$ prostate cancer patients (11 with HRPC and 1 with non-HRPC) and 5 HLA-A2$^+$ healthy volunteers were enrolled in this study after informed consent was obtained. None of these participants were infected with human immunodeficiency virus (HIV). Twenty milliliters of peripheral blood was obtained, and peripheral blood mononuclear cells (PBMCs) were prepared by Ficoll-Conray density-gradient centrifugation. The expression of HLA-A2 molecules on PBMCs of cancer patients and healthy donors was first determined by flow cytometry, and HLA-A2 subtypes were determined by the sequence-specific oligonucleotide probe method.

Cell lines

T2 is an HLA-A*0201-expressing line. PC93 is an HLA-A2-negative prostate cancer cell line (HLA-A*6802$^+$) that was established by K. Ohishi (Department of Urology, Kyoto University, Japan). To establish PC93 cells stably expressing HLA-A2 molecules, pCR3.1 vector (Invitrogen, Calif.), which was inserted with the *HLA-A*0201* gene, was electroporated into PC93 cells and selected with neomycin (Gibco BRL, Grand Island, N.Y.) at a dose of 0.75 mg/ml. An established cell line was designated as PC93-A2. LNCap is a prostate carcinoma cell line. Both colo201 and colo302 are HLA-A2$^+$ and HLA-A2$^-$ colon carcinoma cell lines, respectively. All cell lines were maintained in RPMI-1640 medium (Gibco BRL) supplemented with 10% FCS.

RT-PCR

Total RNA was isolated from cancer cell lines using RNAzol B (Tel-Test, Friendswood, Texas). The cDNA was prepared using the SuperScript Preamplification System for First Strand cDNA Synthesis (Invitrogen, Calif.), and it was amplified using the following primers. The primer pair used for PSCA was as follows:

- Sense primer, 5'-GCAAGAAGAACATCACGTGC-3'
- Antisense primer, 5'-TAGGATGTGCCTCAGGAACC-3'.

The primer pair used for glyceraldehydes 3-phosphate dehydrogenase (GAPDH) was as follows:

- Sense primer, 5'-ACAACAGCCTCAAGATCATCAG-3',
- Antisense primer, 5'-GGTCCACCACTGACACGTTG-3'.

The PCR was performed using Taq DNA polymerase in a DNA thermal cycler (iCycler, Bio-Rad Laboratories, Calif.) for 30 cycles (at 94°C for 1 min, 60°C for 2 min, and 72°C for 1 min).

Flow cytometric analysis

The expression of PSCA on tumor cell lines were examined using anti-PSCA mAb (1G8: mouse IgG1) [6], which was kindly provided by R.E. Reiter (Department of Urology, University of California). To examine the expression of HLA-A2 molecules on tumor cell lines, cells were stained by anti-HLA-A2 mAb, followed by FITC-conjugated goat anti-mouse IgG. The results were analyzed by the CELLQuest program (Becton Dickinson, Calif.).

Peptides

Eleven PSCA-derived peptides, which are listed in Table 1, were prepared based on the HLA-A*0201 binding motif. All peptides were of >90% purity and were purchased from Biologica (Nagoya, Japan). Influenza virus-derived (GILGFVFTL), Epstein-Barr virus (EBV)-derived (GLCTLVAML), and HIV-derived (SLYNTYATL)

Table 1 Reactivity of prostate stem cell antigen (PSCA) peptide-stimulated peripheral blood mononuclear cells (PBMCs) from HLA-A2$^+$ patients with prostate cancer. *EBV* Epstein-Bar virus. *ND* not determined

Peptides	Amino acid sequence	Score[a]	Prostate cancer patients												Total
			1 A0201	2 A0201	3 A0206	4 A0206	5 A0206	6 A0206	7 A0206	8 A0206	9 A0206	10 A0207	11 A0207	12 A0210	
			IFN-γ production (pg/ml)[b]												
PSCA4–13	VLLALLMAGL	309	0	*415*	4	5	19	*629*	20	86	0	25	26	19	2 of 12
PSCA5–13	LLALLMAGL	84	0	7	52	15	39	*136*	66	63	0	*116*	60	14	2 of 12
PSCA7–15	ALLMAGLAL	79	5	0	*101*	50	10	*104*	*121*	*133*	0	*198*	56	19	5 of 12
PSCA21–30	LLCYSCKAQV	118	4	*124*	0	*302*	89	*1342*	0	60	0	*291*	86	2	4 of 12
PSCA43–51	OLGEQCWTA	153	20	0	32	27	10	*274*	43	2	21	24	*131*	22	2 of 12
PCA70–79	CVDDSQDYYV	55	0	54	40	50	16	*253*	25	0	64	74	47	*121*	2 of 12
PSCA106–115	LLALLPALGL	36	0	5	35	34	34	*143*	*116*	27	61	34	*147*	55	3 of 12
PSCA108–117	ALLPALGLLL	79	7	20	46	40	39	28	15	0	50	11	59	46	0 of 12
PWA109–117	LLPALGLLL	36	10	8	63	40	1	58	14	23	36	57	92	93	0 of 12
PSCA14–22	ALQPGTALL	21	0	4	0	5	6	ND	ND	0	0	20	67	0	0 of 10
PSCA105–113	ALLALLPAL	25	0	0	24	29	5	ND	ND	0	17	50	79	17	0 of 10
EBV	GLCTLVAML	–	2	*312*	21	21	17	*142*	44	*847*	0	0	53	*173*	4 of 12
Flu	GILGFVFTL	–	0	0	*554*	22	*292*	*236*	*395*	34	26	*105*	57	*134*	5 of 12

[a]Scores represent the estimated half-time of dissociation of the PSCA peptides binding HLA-A2 molecules

[b]The PBMCs of HLA-A2$^+$ prostate cancer patients were in vitro stimulated with the indicated PSCA peptides, as described in "Materials and methods"

On day 15, the cultured PBMCs were tested for their reactivity to T2 cells, which were pre-pulsed with a corresponding peptide in quadruplicate

Values represent the results of the best of the four wells, and background IFN-γ production in response to the HIV peptide was subtracted

Successful induction of peptide-specific CTLs was judged to be positive when more than 100 pg/ml IFN-γ was produced in response to corresponding peptide-pulsed T2 cells compared with HIV peptide-pulsed T2 cells. Positive responses are italicized

peptides with the HLA-A2 binding motif were used as a control. All peptides were dissolved with dimethyl-sulfoxide at a dose of 10 mg/ml.

Assay for peptide-specific CTLs in PBMCs

The assay for the detection of peptide-specific CTLs was performed according to a previously reported method with several modifications [9]. In brief, PBMCs (1×10^5 cells/well) were incubated with 10 μg/ml of each peptide in a U-bottom-type 96-well microculture plate (Nunc, Roskilde, Denmark) in 200 μl of culture medium. The culture medium consisted of 45% RPMI 1640, 45% AIM-V medium (Gibco BRL), 10% FCS, 50 U/ml of interleukin-2 (IL-2), and 0.1 mM MEM nonessential amino acid solution (Gibco BRL). Half of the culture medium was removed and replaced with the new medium containing a corresponding peptide (20 μg/ml) every 5 days. On the fifteenth day of culture, the harvested cells were tested for their ability to produce interferon (IFN)-γ in response to T2 cells (1×10^4 cells/well), which were pre-loaded with either a corresponding peptide or the HIV peptide as a negative control. After an 18-h incubation, the supernatant was collected, and the level of IFN-γ was determined by enzyme-linked immunosorbent assay (ELISA; limit of sensitivity: 10 pg/ml).

A small number of PBMCs (1×10^5/well) were applied in microwell plates, and were separately stimulated with corresponding peptides in an assay for peptide-specific CTLs. Under these conditions, the reactivity varied considerably among individual wells, as reported previously [9]; therefore, each well was separately estimated to screen for the presence of peptide-specific CTL precursors.

In vitro culture for CTL assay

The PBMCs from patients (1×10^5 cells/well) were incubated with 10 μg/ml of each peptide in a U-bottom-type 96-well microculture plate (Nunc, Roskilde, Denmark) at a volume of 200 μl of the culture medium containing 50 U/ml of IL-2. Half of the culture medium was removed and replaced with the new medium containing a corresponding peptide (20 μg/ml) and IL-2 (50 U/ml) every 5 days. On the fifteenth day of culture, half of the cultured cells were harvested, washed, and divided into three wells. The cultured cells in each well were cultured with or without PC93 or PC93-A2 cells (1×10^4/well). After an 18-h culture, the supernatants were collected, and the levels of IFN-γ were determined by ELISA. Then, the cells in the wells producing IFN-γ in an HLA-A2-restricted manner were collected and further cultured in order to obtain a sufficiently large number of cells to carry out a CTL assay.

Cytotoxicity assay

Cultured cells were tested for cytotoxicity against both PC93 and PC93-A2 by a 6-h ^{51}Cr-release assay.Two thousand ^{51}Cr-labeled cells per well were cultured with effector cells in 96 round-well plates at the indicated effector/target ratios. The specific ^{51}Cr-release was calculated according to the following formula:

$$\frac{\text{test c.p.m.} - \text{spontaneous c.p.m.}}{\text{test c.p.m.} - \text{spontaneous c.p.m.}} \times 100$$

Spontaneous release was determined by the supernatant of the sample incubated with no effector cells, and the total release was then determined by the supernatant of the sample incubated with 1% Triton X (Wako Pure Chemical Industries, Osaka, Japan). In some experiments, $CD8^+$ T cells were positively isolated using CD8 Positive Isolation Kit (Dynal, Oslo, Norway) from the PSCA peptide-stimulated PBMCs. The positive percentage of $CD8^+$ T cells was >97%. In other experiments of cytotoxicity assay, 10 μg/ml of either anti-HLA class I (W6/32: mouse IgG2a) or anti-HLA-DR (L243: mouse IgG2a) mAb was added into wells at the initiation of the culture.

Cold inhibition assay

The specificity of PSCA peptide-stimulated CTLs was confirmed by a cold inhibition assay. In brief, ^{51}Cr-labelled target cells (2×10^3 cells/well) were cultured with the CTLs (2×10^4 cells/well) in 96 round-well plates with 4×10^4 cold target cells. T2 cells, which were pre-pulsed with either the HIV peptide or the corresponding PSCA peptide, were used as cold target cells.

Detection of peptide-specific immunoglobulin G

Peptide-specific immunoglobulin G (IgG) levels in plasma were measured by ELISA, as previously reported [22, 23]. Briefly, a peptide (20 μg/well)-immobilized plate was blocked with Block Ace

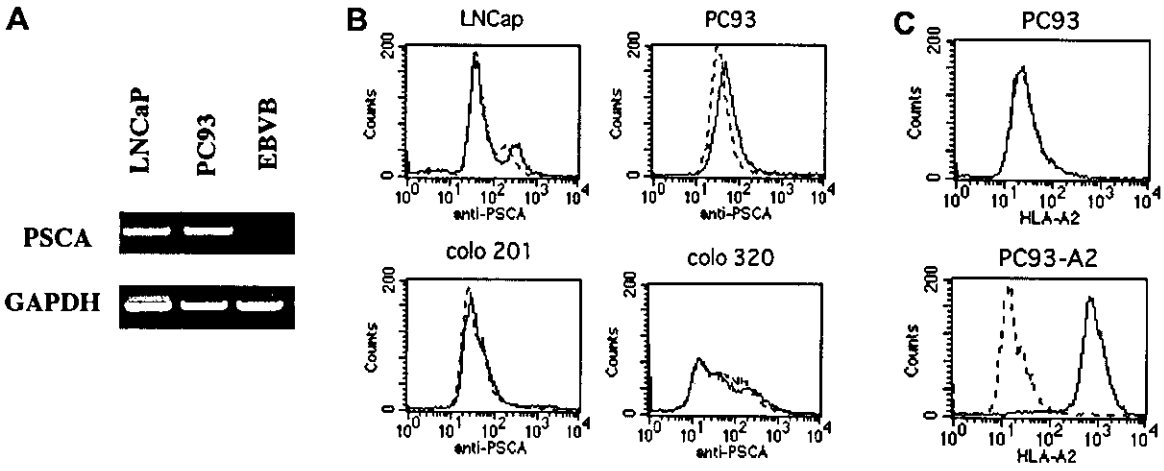

Fig. 1A–C Expression of prostate stem cell antigen (*PSCA*) on PC93 cells and an HLA-A2-expressing transfectant cell line. **A** RT-PCR was carried out using cDNA from either LNCaP, PC93, or Epstein-Barr virus (EBV)-transformed B cells. Thirty PCR cycles were carried out. *GAPDH* glyceraldehyde 3-phosphate dehydrogenase. **B** Flow cytometric analysis was performed on LNCap, PC93, colo201, and colo320 cells. These cells were stained with anti-PSCA mAb, followed by FITC-conjugated anti-mouse IgG mAb. The *dashed lines* represent the staining without the first mAb. **C** Flow cytometric analysis was performed on PC93 and PC93-A2 cells. These cells were stained with anti-HLA-A2 mAb, followed by FITC-conjugated anti-mouse IgG mAb. The *dashed lines* represent the staining without the first mAb

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A phase I trial of cytotoxic T-lymphocyte precursor-oriented peptide vaccines for colorectal carcinoma patients

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In most protocols of peptide-based vaccination, no consideration has been paid to whether or not peptide-specific cytotoxic T-lymphocyte (CTL) precursors are pre-existent in cancer patients. Initiation of immune boosting through vaccination is better than that of immune priming to induce prompt and strong immunity. In this study, 10 human histocompatibility leukocyte antigen-A24$^+$ patients with advanced colorectal carcinomas were treated with up to four peptides that had been positive for pre-vaccination measurement of peptide-specific CTL precursors in the circulation (CTL precursor-oriented peptide vaccine). No severe adverse effect was observed, although local pain and fever of grade I or II were observed. Post-vaccination peripheral blood mononuclear cells (PBMCs) from five patients demonstrated an increased peptide-specific immune response to the peptides. Increased CTL response to cancer cells was detected in post-vaccination PBMCs of five patients. Antipeptide immunoglobulin G became detectable in post-vaccination sera of seven patients. Three patients developed a positive delayed-type hypersensitivity response to at least one of the peptides administrated. One patient was found to have a partial response; another had a stable disease, sustained through 6 months. These results encourage further development of CTL precursor-oriented vaccine for colorectal cancer patients.
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Recent advances in molecular biology and cellular immunology have resulted in identification of various antigens and epitopes recognised by human histocompatibility leukocyte antigen (HLA)-class-I-restricted cytotoxic T lymphocytes (CTLs) from various cancers (Bruggen *et al*, 1991; Brichard *et al*, 1993; Kawakami *et al*, 1994a, b Fisk *et al*, 1995; Peoples *et al*, 1995; Robbins *et al*, 1996; Correale *et al*, 1997; Correale *et al*, 1998). Many clinical trials of peptide-based immunotherapy have shown an increased immune response to the vaccinated peptides (Rosenberg *et al*, 1998; Marchand *et al*, 1999; Jager *et al*, 2000; Gajewski *et al*, 2001; Gjertsen *et al*, 2001; Lau *et al*, 2001; Valmori *et al*, 2001). However, these initial clinical studies have produced few clinical responses in the various types of cancer treated including melanoma and colorectal cancer (Finn and Lotze, 2001; Miyagi *et al*, 2001; Sadanaga *et al*, 2001). This failure could be in part due to the insufficient and late stages of CTL induction by the current regimen, in which pre-vaccination screening of suitable peptides for each patient among a large number of candidate peptides is not conducted. As a result, vaccination in the present study began with immune priming. This standard regimen could be effective in preventing infectious disease. However, the main goal of a cancer vaccine is treatment of malignant disease. The time-lag necessary for priming of an antitumour response should be seriously considered, as the expected survival of most advanced cancer patients under these regimens is 3–9 months (Cole and Rodu, 2001; Miyagi *et al*, 2001). Therefore, the development of a new regimen of therapeutic vaccine is needed (Finn and Lotze, 2001). One regimen might include pre-vaccination measurement of peptide-specific CTL precursors in the circulation, followed by vaccination of only CTL-reactive peptides (CTL precursor-oriented vaccine). We have previously reported 14 vaccine candidate peptides that can induce HLA-A24-restriced and tumour-specific CTL in cancer patients (Gomi *et al*, 1999; Kikuchi *et al*, 1999; Yang *et al*, 1999; Harashima *et al*, 2000; Kawano *et al*, 2000; Nakao *et al*, 2000; Nishizaka *et al*, 2000). We have also shown that most cancer patients have peptide-specific CTL precursors for some of these peptides, and that peripheral blood mononuclear cells (PBMCs) stimulated with positive peptides show HLA-class-I-restricted and tumour-specific cytotoxicity (Hida *et al*, 2002; Maeda *et al*, 2002; Suzuki *et al*, 2002). In the present study, patients with advanced stages of colorectal cancer were immunised with up to four peptides identified in pre-vaccination measurement of peptide-specific CTL precursors to evaluate the toxicities and responses to CTL precursor-oriented peptide vaccination.

MATERIALS AND METHODS

Patients and eligibility criteria

The study protocol was approved by the Institutional Ethical Review Boards of Hokkaido University and Kurume University, respectively. Complete written informed consent was obtained

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from all of patients at the time of enrolment. According to the protocol, patients were required to be HLA-A24 positive, and to have a histologically confirmed lesion of colorectal carcinoma. Eligibility criteria included an age of 85 years or less, serum creatinine of less than 1.4 mg dl^{-1}, bilirubin of less than 1.5 mg dl^{-1}, a platelet count of 100 000 μl^{-1} or more, haemoglobin of 8.0 g dl^{-1} or more, and total WBC of 3000 μl^{-1} or more. Hepatitis B surface antigen, Hepatitis C antigen, and human immunodeficiency virus (HIV) were required to be negative. The patients, who had been untreated for at least 4 weeks before the study, had an Eastern Cooperative Oncology Group performance status of 0–2. The treatment was carried out at the First Department of Surgery, Hokkaido University School of Medicine (patients 1–9) and the Department of Surgery, Kurume University School of Medicine (patient 10) from November 2000 through April 2002. All immunological analyses were carried out at the Department of Immunology, Kurume University School of Medicine.

Screening of peptide-specific CTL precursors

A volume of 30 ml of peripheral blood was obtained pre- and post-(3rd, 6th, and 9th) vaccination, and PBMCs were isolated by means of Ficoll–Conray density gradient centrifugation, as reported previously (Miyagi *et al*, 2001). A previously reported method was used to detect peptide-specific CTL precursors in PBMCs (Hida *et al*, 2002; Suzuki *et al*, 2002). Briefly, PBMCs (1×10^5 cells $well^{-1}$) were incubated with 10 μM of a peptide in wells of u-bottom-type 96-well microculture plates (Nunc, Roskilde, Denmark) in 200 μl of culture medium. The culture medium consisted of 45% RPMI-1640 medium, 45% AIM-V® medium (GIBCO BRL), 10% FCS, 100 U ml^{-1} of interleukin (IL)-2, and 0.1 μM MEM nonessential amino-acid solution (GIBCO BRL). Every 3 days, half of the medium was removed and replaced with new medium containing a corresponding peptide (20 μM). The assay was performed in quadruplicate. After a 12-day incubation, the cultured cells in one well were divided into four wells, two of which were used for corresponding peptide-pulsed C1R-A2402 cells, and the other two of which were used for HIV peptide (RYLRQQLLGI)-pulsed C1R-A2402 cells. The HLA-A24-binding HIV peptide was used as a negative control. After an 18-h incubation period, the supernatants were collected and the level of interferon (IFN)-γ was determined by enzyme-linked immunosorbent assay (ELISA) (limit of sensitivity: 10 pg ml^{-1}). The background response to HIV peptide-pulsed C1R-A2402 cells was subtracted from the value. Assessment of peptide-specific CTL precursors was performed based on two parameters, the *P*-value by the Student's *t*-test and IFN-γ production, as described in legends of Tables 2 and 4. According to the results of this test, up to four positive peptides were selected for each patient, utilised in a skin test, and then, if negative, injected as a vaccination. The screening of peptide-specific CTL precursors was performed by the same method after the 6th vaccination to evaluate the effects of immunisation.

Peptides and vaccination

The peptides utilised in the present study were prepared under conditions of Good Manufacturing Practice using a Multiple Peptide System (San Diego, CA, USA). Montanide ISA-51, an incomplete adjuvant, was manufactured by Seppic, Inc (Franklin Lakes, NJ, USA). The peptides were supplied in vials containing 3 mg ml^{-1} sterile solution for injection. A 3 mg portion of peptide with sterile saline was added in a 1 : 1 volume to Montanide ISA-51, then mixed in a Vortex mixer (Fisher, Inc, Alameda, CA, USA). The resulting emulsion was injected subcutaneously into the lateral thigh using a glass syringe. Patients were vaccinated every 14 days for a total of three injections to measure the toxicity. For the patients with no toxicity, the vaccinations were repeated biweekly up to 15 times with informed consent from each patient.

Delayed-type hypersensitivity (DTH) skin test

Skin tests were performed using 50 μg of each peptide injected intradermally in a volume of 100 μl using a tuberculin syringe and a 27-gauge needle. Saline was a negative control for assessment of DTH. At least 7 mm of induration or erythema read 48 h after injection was needed to score the skin test as positive (Nestle *et al*, 1998).

Assay of cytotoxicity

Cytotoxic activity was measured using a standard 6-h ^{51}Cr-release assay (Gomi *et al*, 1999; Miyagi *et al*, 2001). Cryopreserved PBMCs were thawed and cultured in the medium consisting of 45% RPMI-1640 medium, 45% AIM-V® medium (GIBCO BRL), 10% FCS, 100 U ml^{-1} of IL-2, and 0.1 μM MEM nonessential amino-acid solution (GIBCO BRL). On the 14th day of culture, the cells were harvested and served for the assay. To avoid the bias of bioassays, PBMCs harvested at different times from a single patient were thawed at the same time. SW620 (HLA-A24$^+$, colon adenocarcinoma), COLO201 (HLA-A24$^-$, colon adenocarcinoma), and PHA-blastoid T cells (HLA-A24$^+$) were used as target cells (1×10^3 $well^{-1}$), and 50-fold unlabelled K562 cells were added into wells to decrease nonspecific killing activity.

Purification of CD8$^+$ or CD4$^+$ T cells

CD8$^+$ or CD4$^+$ T cells were positively isolated from peptide-stimulated PBMCs using the CD8 Positive Isolation Kit (DYNAL, Oslo, Norway) or the CD4 Positive Isolation Kit (DYNAL) according to the manufacturer's instructions. In both cases, the percentage of purified CD8$^+$ or CD4$^+$ T cells was more than 90% (data not shown).

Kinetics of peptide-specific CTL precursors

To detect the kinetics of peptide-specific CTL precursor frequency in patient 1, PBMCs from before and after vaccination were incubated at 100 cells per well of a 96-well u-bottom microculture plate in the presence of feeder cells without the peptides. Cells from each well were harvested on the 14th day of culture and tested for their ability to produce IFN-γ by recognition of peptide-pulsed CIR-A2402 cells in duplicate assay. The well was considered positive if it contained effector cells producing much higher than 100 pg ml^{-1} and also statistically significant levels ($P < 0.05$ by the Student's *t*-test) of IFN-γ in response to CIR-A2402 cells preloaded with a corresponding peptide as compared with those in response to the HIV peptide-pulsed CIR-A2402 cells.

Detection of serum immunoglobulin G (IgG) levels

An ELISA was used to detect the serum IgG levels specific to the peptides administered, as reported previously (Miyagi *et al*, 2001). Briefly, the peptide (20 μg $well^{-1}$)-immobilised plate was blocked with Block Ace (Yukijirushi, Tokyo, Japan), washed, and 100 μl $well^{-1}$ of serum samples diluted with 0.05% Tween 20-Block Ace were added to the plate. After a 2-h incubation, the plate was washed and further incubated for 2 h with an 1 : 1000-diluted rabbit anti-human IgG (DAKO, Glostrup, Denmark). The plate was washed, after which 100 μl of 1 : 100-diluted goat anti-rabbit Ig-conjugated horseradish peroxidase-dextran polymer (En Vision, DAKO) was added to each well, and the plate was incubated for 40 min. After washing, 100 μl $well^{-1}$ of tetramethyl-benzidine substrate solution (KPL, Guildford, UK) was added, and the reaction was stopped by the addition of 1 M phosphoric acid. To estimate peptide-specific IgG levels, the optical density values of each sample were compared with those of serially diluted standard samples, and the values are shown as the optical density. The

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specificity of the ELISA was tested as follows. Serum samples (1:100 diluted) were incubated in wells precoated with corresponding or irreverent peptides to absorb the peptide-specific IgG. After a 2-h incubation at room temperature, samples were transferred to new wells precoated with the sample peptide used in the first absorption, and this protocol was repeated for a total of three times. The samples were then subjected to peptide-specific IgG ELISA.

Evaluation of treatment response

All known sites of disease were evaluated by CT-scan or X-ray examination pre- and post-vaccinations (the 3rd, 6th, 9th, and 12th). Patients were assigned a response category according to the response evaluation criteria in solid tumours, a revised version of the WHO criteria published in the WHO Handbook for reporting results of cancer treatment in June 1999.

RESULTS

Demographics of the patients

In all, 10 patients with advanced colorectal carcinomas were enroled in this phase I study. Demographic details of the patients are shown in Table 1. The median patient age was 67 years (range 28–78). All patients had liver, lung, peritoneum, or lymph node metastases. All patients underwent surgical resection of the primary lesion; nine had failed previous chemotherapy. All 10 patients completed the first three vaccinations within the protocol under informed consent, and all of them received more vaccinations (6–15) under additional informed consent.

Screening of peptide-specific CTL precursors

All tumour-related antigens were identified by screening of a cDNA library from tumour cells using tumour-reactive CTLs. The peptides used for this study have the potential to induce HLA-A24-restricted and tumour-specific CTL activity in PBMCs of HLA-A24$^+$ cancer patients (Kikuchi *et al*, 1999; Yang *et al*, 1999; Gomi *et al*, 1999; Harashima *et al*, 2000; Kawano *et al*, 2000; Nakao *et al*, 2000; Nishizaka *et al*, 2000). Pre-vaccination PBMCs were provided for screening of the CTL precursors reactive to the 14 candidate peptides, followed by selection of peptides based on evaluation of the results with the criteria shown in Table 2. The assay was performed in quadruplicate. After a 12-day incubation, the cultured cells in one well were separated into four wells, two of which were used for corresponding peptide-pulsed CIR-A2402 cells, and the other two of which were used for HIV peptide-pulsed CIR-A2402 cells. The HLA-A24-binding HIV peptide was used as a negative control. The assessment of peptide-specific CTL

Table 1 Patient characteristics

Patient	Age	Sex	Primary site	Metastasis site	Previous treatment[a]	P.S.[b]	No. of vaccination received[c]
1	67	M	Transverse colon	Liver, para-aortic lymph node	S, C	0	15
2	38	F	Transverse colon	Liver, peritoneum (intrapelvic)	S, C	0	12
3	67	M	Ascending colon	Lung, liver	S, C	1	6
4	78	M	Rectum	Liver	S, C, M	0	10
5	70	F	Sigmoid colon	Lung, liver	S, C	1	11
6	72	M	Rectum	Liver	S	1	10
7	67	M	Sigmoid colon	Lung	S, C	0	8
8	44	M	Sigmoid colon	Lung, liver	S, C	0	6
9	46	M	Ascending colon	Peritoneum (intrapelvic)	S, C	0	6
10	28	F	Sigmoid colon	Peritoneum (intrapelvic)	S, C	1	8

[a]S = surgery; C = chemotherapy; M = percutaneous microwave coagulation therapy. [b]P.S. = performance status by ECOG score. [c]The initial protocol consisted of three vaccinations; additional vaccinations were subsequently performed to patients who showed a favourable clinical course after they provided further informed consent.

Table 2 Pre-vaccination screening of peptide-specific CTL precursors

		Patients											
Peptide	Sequence	1	2	3	4	5	6	7	8	9	10	Positive case	Vaccinated case
SART1 690	EYRGFTQDF								OA	OAA		2/10	2/10
SART2 93	DYSARWNEI								OAB			1/10	1/10
SART2 161	AYDFLYNYL								OA	OAA		2/10	2/10
SART2 899	SYTRLFLIL	●B										1/10	0/10
SART3 109	VYDYNCHVDL	OA	OB			OC	OC	OA		OA		6/10	6/10
SART3 315	AYIDFEMKI							OC			OC	2/10	2/10
CyB 84	KFHRVIKDF							●A				1/10	0/10
CyB 91	DFMIQGGDF			OA	OA			OC			OA	4/10	4/10
lck 208	HYTNASDGL	OC	OA		OA	OC		OC	OB		OAAA	7/10	7/10
lck 488	TFDYLRSVL		OAB		OAAAA					OAB	OA	4/10	4/10
lck 488	DYLRSVLEDF	OA		OA	OAA							3/10	3/10
ART1 170	EYCLKFTKL	●B			●A		●A			●AA		4/10	0/10
ART4 13	AFLRHAAL					●C						1/10	0/10
ART4 75	DYPSLSATDI			OA			OB					2/10	2/10

White circles indicate that the peptide was positive for the CTL precursor induction assay and was injected. Black circles indicate that the peptide was positive for the CTL precursor induction assay but was not administrated due to immediate-type hypersensitivity by skin test. The assay was performed in quadruplicate and the background response to the HIV peptide was subtracted from the value. The result was evaluated by the following criteria: A, $P<0.05$ and IFN-γ production $>50\,pg\,ml^{-1}$; B, $P<0.05$ and $50\,pg\,ml^{-1}>$IFN-γ production $>25\,pg\,ml^{-1}$; C, $0.05<P<0.1$ and IFN-γ production $>25\,pg\,ml^{-1}$. The classification is shown by letters of the alphabet, and each character represents the results of each well. For example, ABC means that three wells were judged as A, B, and C, and one well was negative.

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precursors was performed based on two parameters, the *P*-value by the Student's *t*-test and IFN-γ production, as shown in the table legend. When these peptides were found to induce immediate-type hypersensitivity by a skin test, a fifth peptide was vaccinated if it proved negative in the skin test. $SART2_{899}$, CyB_{91}, $ART1_{170}$, and $ART4_{13}$ were positive for immediate-type hypersensitivity in all patients tested and were not injected at all. As a result, five patients were injected with four peptides, three patients with three peptides, and two with two peptides. The vaccinated peptides for each patient are shown in Table 2. It is noteworthy that the profiles of the vaccinated peptides varied greatly among the 10 patients.

Toxicities

All 10 patients were evaluated for toxicity; the overall toxicities are shown in Table 3. The vaccinations were generally well-tolerated, but almost all patients (eight out of 10) had grade I or II local redness and swelling at the injection sites. Fever with mild flu-like symptoms was observed in four patients (grade I or grade II), although this symptom was transient and no medication was needed. Grade I fatigue or nausea was observed in two patients, and grade I anorexia, diarrhoea, or vomiting was observed in one. No vaccine-related grade III or IV toxicity was observed (data not shown). There was no clinical evidence of an autoimmune reaction as determined by symptoms, physical examination, or laboratory test.

Cellular immune responses

Post-vaccination (6th) PBMCs showed increased amounts of peptide-specific IFN-γ production compared to pre-vaccination PBMCs in five out of 10 patients (1, 2, 5, 6, and 10), as described in Table 4. Representative results of patients 1 and 2 are shown in Figure 1A. In patients 1 and 2, CTL response to the lck_{208} was apparently induced after the 6th vaccination. In five other patients, peptide-specific CTL response decreased. We further tested the reactivity of purified $CD4^+$ or $CD8^+$ T cells in response to the administered peptides. The pre- or post-6th vaccination PBMCs from patient 2 were *in vitro* stimulated, and purified $CD4^+$ or $CD8^+$ T cells were tested for their reactivity to the $SART3_{109}$ peptide-pulsed C1R-A2402 cells. As shown in Figure 1B, purified $CD8^+$ T cells from the post-vaccination PBMCs of patient 2 produced IFN-γ in an antigen-specific manner, although no definite IFN-γ production specific to the $SART3_{109}$ peptide was observed when unseparated post-6th PBMCs from patient 2 were used (Figure 1A). Purified $CD4^+$ T cells failed to produce IFN-γ in a peptide-specific manner. On the other hand, no peptide-specific IL-4 production was observed in the case with purified $CD8^+$ or $CD4^+$ T cells (data not shown).

We next examined cytotoxicity of pre- and post- (3rd, 6th, and 9th) vaccination PBMCs from eight patients against SW620 (HLA-$A24^+$ colon tumour cells), COLO201 cells (HLA-$A24^-$ colon tumour cells), and PHA-activated T cells (HLA-$A24^+$) (Figure 2). Tumour-related antigens from which all peptides used in this study were derived are nonmutated self-antigens overexpressed in tumour cells, including SW620 and COLO201 (Shichijo *et al*, 1998; Yang *et al*, 1999; Gomi *et al*, 1999; Harashima *et al*, 2000; Kawano

Table 3 Toxicities associated with the peptide vaccination

Toxicities	Grade* I	Grade* II	Total
Anorexia	1		1
Dermatologic	7	1	8
Diarrhoea	1		1
Fatigue	2		2
Fever	2	2	4
Nausea	2		2
Vomiting	1		1

*Toxicities are based on the NIH Common Toxicity Criteria.

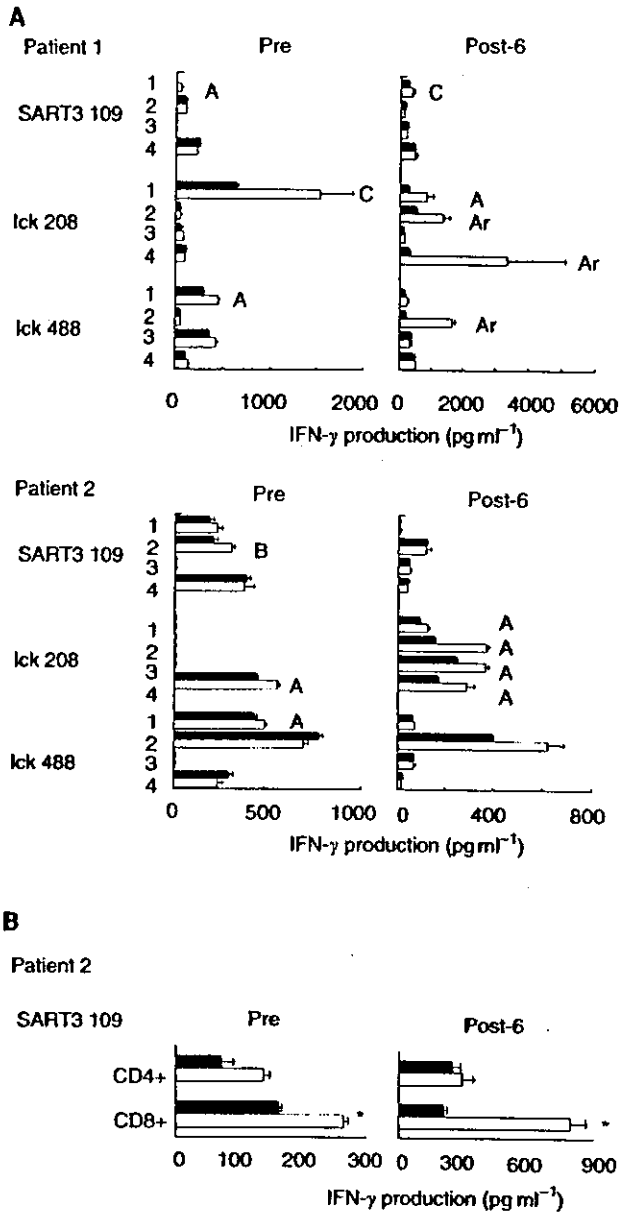

Figure 1 Assay of peptide-specific CTL precursors. (**A**) Pre- and post-(6th) vaccination PBMCs were provided for screening of reactivity to each of the 14 peptides listed in Table 2 in the quadruplicate assays. Representative results of patients 1 and 2 are shown in this figure. The peptide-stimulated PBMCs were cultured with C1R-A2402 cells that were preloaded with the corresponding peptide (open bar) or the HIV peptide (closed bar). The level of IFN-γ in the supernatant was determined by ELISA. The result was evaluated by the classification shown in the legend of Table 4. Each alphabet character represents the result of each well. (**B**) Pre- and post- (6th) vaccination PBMCs from patient 2 were stimulated *in vitro* with the $SART3_{109}$ peptide. The peptide-stimulated PBMCs were harvested, and positively isolated $CD4^+$ or $CD8^+$ T cells were cultured in triplicate with C1R-A2402 cells that were preloaded with the $SART3_{109}$ peptide (open bar) or the HIV peptide (closed bar). The level of IFN-γ in the supernatant was determined by ELISA. *Statistically significant at $P<0.05$.

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Table 4 Summary of response to the peptide vaccination

Patient	Peptide	Peptide-specific CTL[a] Pre	Peptide-specific CTL[a] Post	Ab to peptide Pre	Ab to peptide Post[b]	DTH Pre	DTH Post[b]	Clinical response/ Time to progression (months)
1	SART3 109	A	C	–	–	–	7 mm (9)	PR/7<
	lck 208	C	ArArA	–	+ (6)	–	10 mm (6)	
	lck 488	A	Ar	–	+ (9)	–	10 mm (6)	
2	SART3 109	B	–	–	+ (6)	–	–	SD/7
	lck 208	A	AAAA	–	–	–	–	
	lck 486	AB	A	–	+ (9)	–	–	
3	CyB 91	A	C	–	–	–	–	PD/3
	lck 488	A	A	–	–	–	–	
	ART4 75	A	–	–	–	–	–	
4	CyB 91	A	–	–	–	–	–	PD/3
	lck 208	A	C	–	–	–	7 mm (3)	
	lck 486	AAAA	C	–	–	–	7 mm (3)	
	lck 488	AA	–	–	–	–	7 mm (3)	
5	SART3 109	C	ArB	–	–	–	–	PD/4
	lck 208	C	A	–	+ (9)	–	–	
6	SART3 109	C	ArBC	–	+ (6)	–	–	PD/3
	ART4 75	B	B	–	–	–	–	
7	SART3 109	A	A	–	+ (6)	–	10 mm (3)	PD/4
	SART3 315	C	–	–	–	–	–	
	CyB 91	C	–	–	–	–	–	
	lck 208	C	C	–	–	–	–	
8	SART1 690	A	–	–	–	–	–	PD/4
	SART2 93	AB	–	–	–	–	–	
	SART2 161	A	C	–	–	–	–	
	lck 208	B	–	–	–	–	–	
9	SART1 690	AA	–	–	–	–	–	PD/4
	SART2 161	AA	–	–	–	–	–	
	SART3 109	A	–	–	–	–	–	
	lck 486	AB	C	–	+(6)	–	–	
10	SART3 315	C	ArAA	–	+(6)	–	–	PD/3
	CyB 91	A	–	–	–	–	–	
	lck 208	AAA	–	–	–	–	–	
	lck 486	AA	–	–	–	–	–	

[a]The CTL precursor induction assay was performed in quadruplicate, and the background response to the HIV peptide was subtracted from the value. The result was evaluated by the following criteria: Ar (armed response), $P<0.1$ and IFN-γ production $>500\,pg\,ml^{-1}$; A, $P<0.05$ and IFN-γ production $>50\,pg\,ml^{-1}$; B, $P<0.05$ and $50\,pg\,ml^{-1}$ >IFN-γ production $>25\,pg\,ml^{-1}$; C, $0.05<P<0.1$ and IFN-γ production $>25\,pg\,ml^{-1}$. The classification is shown by letters of the alphabet, and each character represents the results of each well. For example, ArBC means that three wells were judged as Ar, B, and C, and one well was negative. [b]The number in the parenthesis represents the vaccination when anti-peptide IgG or DTH was detected for the first time.

et al, 2000; Nakao *et al*, 2000; Nishizaka *et al*, 2000). As shown in four cases of patients 2, 3, 7, and 9, cytotoxicity against HLA-A24$^+$ SW620 increased after peptide vaccination compared to that against HLA-A24$^-$ COLO201. In the other four cases, no definite increase in cytotoxicity was observed after the peptide vaccination. No cytotoxicity against HLA-A24$^+$ PHA-blastoid T cells was detected in any case. These results indicate that the peptide vaccination resulted in augmented CTL activity in four out of eight patients.

Serum IgG specific to peptides

No IgG reactive to any of the vaccinated peptides was detected in pre-vaccination sera from any of the 10 patients (Table 4). Significant levels of anti-peptide IgG reactive to SART3- or lck-derived peptides became detectable in the post-vaccination sera of seven patients. Detail results are shown in Figure 3. In patient 1, IgGs reactive to the lck_{208} and the lck_{488} peptides were induced after the 6th and 9th vaccinations, respectively (Figure 3A). A similar result was observed in six other patients (Figure 3B). It is noteworthy that three patients who showed strong peptide-specific CTL response (criteria Ar) after the peptide vaccination were also positive for antipeptide IgG to the corresponding peptides (patient 1 for lck_{208} and lck_{488}, patient 6 for $SART3_{109}$, and patient 10 for $SART3_{315}$). In addition, the IgG response to the lck_{208} peptide in post-vaccination sera of patient 1 was neutralised by absorption with a corresponding peptide, but not with the lck_{408} peptide, whereas the opposite was observed in the case of the IgG response to the lck_{488} peptide (Figure 3C). This peptide-specific absorption demonstrates the validity of the ELISA system.

DTH skin test

No DTH reaction against peptides was observed before vaccination in any patient, while peptide-specific DTH reactions were observed

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