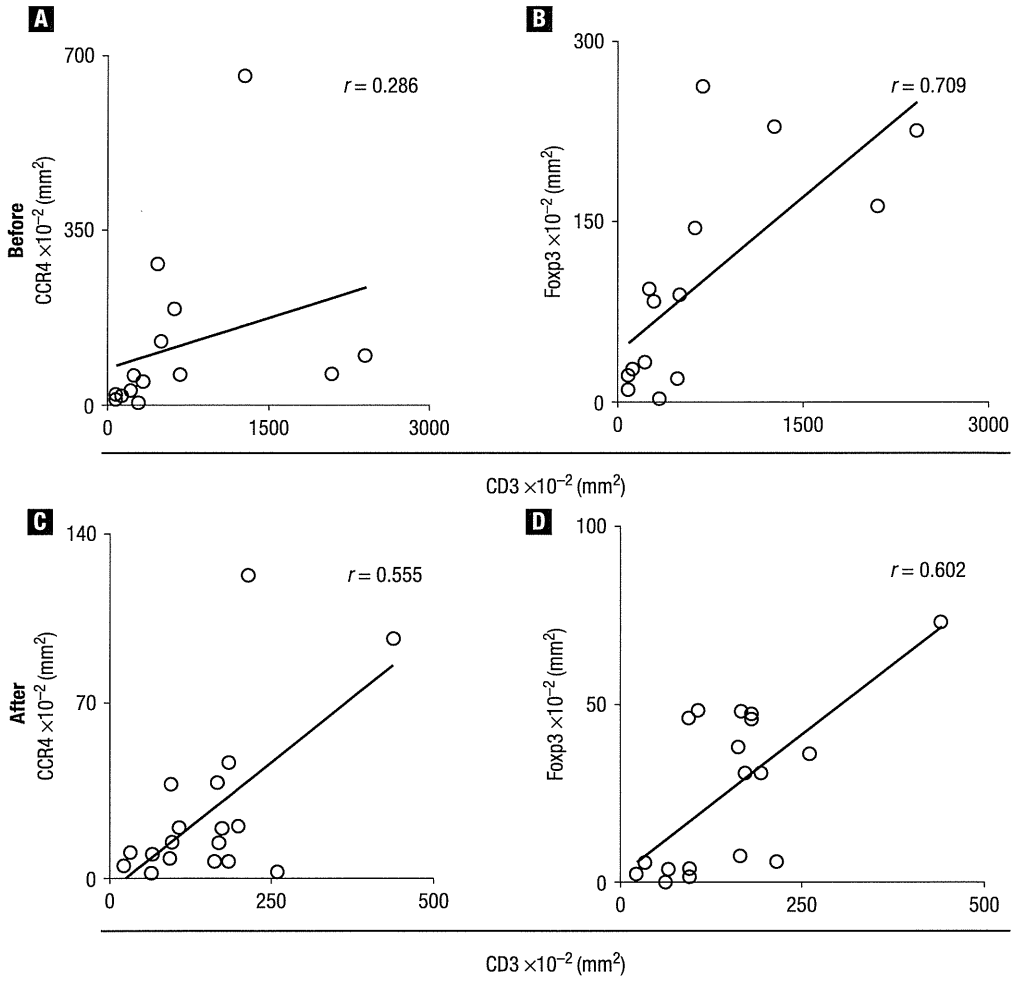
Figure 4 Correlation Between the Lymphocytes Before (A and B) and After (C and D) Bath–Psoralen Plus Ultraviolet A (PUVA) Therapy. There was No Significant Correlation Between CD3 and CCR4 Before Bath-PUVA Therapy, But There was a Positive Correlation After Bath-PUVA Therapy. Correlation Coefficients Were Determined by Spearman Rank Correlation Analyses



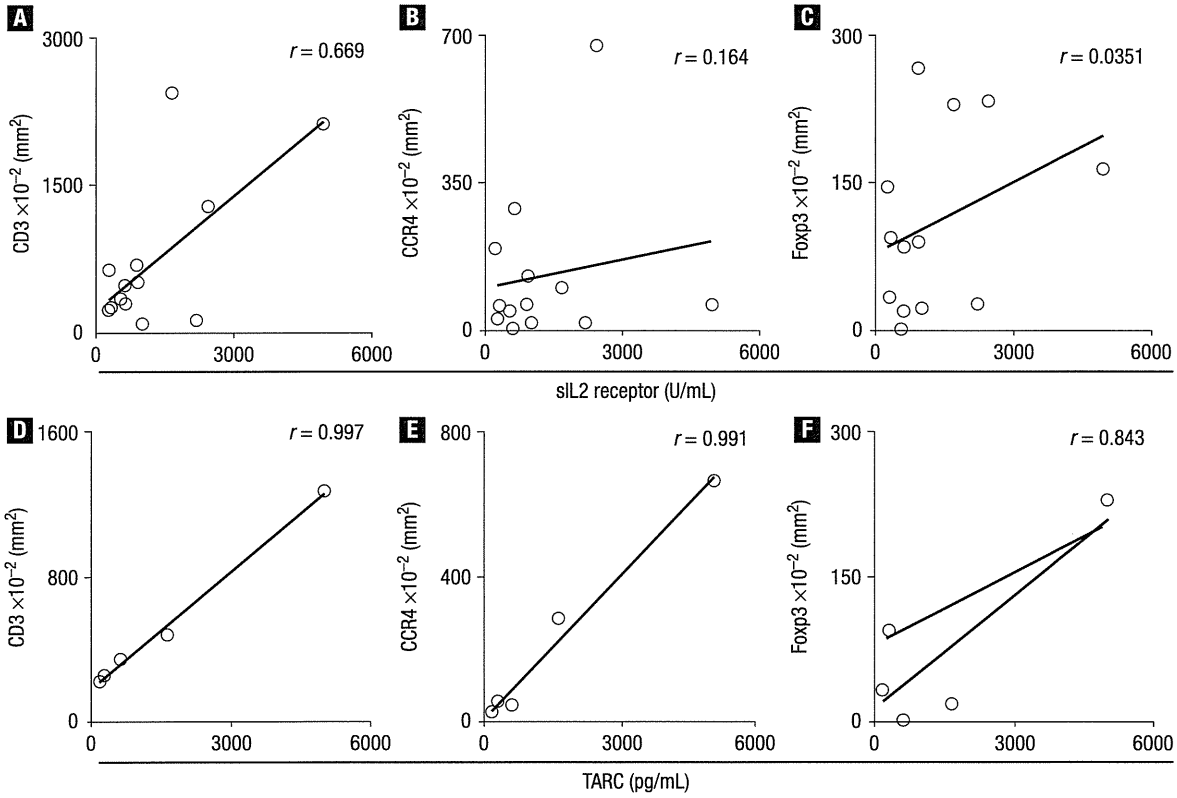
lymphocytes. Therefore we analyzed the numbers of whole stained lymphocytes, including both tumor and reactive cells (Figures 1 and 2). After bath-PUVA therapy, patients with stage I MF had a significant decrease in the number of infiltrating $CD3^+$, $CCR4^+$, and $Foxp3^+$ cells compared with the number of cells before therapy (Figure 3). The number of $CCR4^+$ cells in the lesion significantly decreased from 105.1 ± 164.8 cells/10^{-2} mm^2 to 31.4 ± 39.0 cells/10^{-2} mm^2. Similarly, Tregs in the lesion decreased from 78.1 ± 67.8 cells/10^{-2} mm^2 to 24.7 ± 25.0 cells/10^{-2} mm^2.

Before bath-PUVA, there was no significant correlation between the number of $CD3^+$ cells and $CCR4^+$ cells ($r = 0.286$) (Figure 4A), but after bath-PUVA, the levels of these cells were positively correlated ($r = 0.555$) (Figure 4C). The correlations between the number of $CD3^+$ and $Foxp3^+$ cells were significant before and after bath-PUVA therapy ($r = 0.709$; $r = 0.602$ (Figures 4B and D).

TARC/CCL17 is a CCR4 ligand used as a disease activity marker in atopic dermatitis,[14] and serum TARC/CCL17 levels are also correlated with MF disease activity.[15] Thus the correlation between the number of each type of infiltrating lymphocyte and sIL2R (n = 13) and TARC/CCL17 (n = 5) was evaluated (Figure 5). sIL2R levels were significantly correlated with the number of infiltrating $CD3^+$ cells ($r = 0.669$) but not with the number of infiltrating $CCR4^+$ and $Foxp3^+$ cells ($r = 0.164$ and $r = 0.351$, respectively). TARC/CCL17 levels were significantly correlated with the numbers of infiltrating $CD3^+$, $CCR4^+$, and $Foxp3^+$ cells ($r = 0.997$, $r = 0.991$, and $r = 0.843$, respectively) (Figure 5).

We then assessed the percentages of $CD4^+CD25^+Foxp3^+$ Tregs in $CD4^+$ T cells in PBMCs obtained from patients with MF and healthy volunteers. There was no significant difference between patients with MF and healthy volunteers. Bath-PUVA

Figure 5 Correlation Between Infiltrating Lymphocytes and Soluble IL-2 Receptor (sIL2) Levels (A-C) and Between Infiltrating Lymphocytes and Thymus and Activation-Regulated Chemokine (TARC) Levels (D-F). Soluble Interleukin-2 Receptor (sIL2) Levels Correlated Significantly With CD3, and TARC Levels Correlated Significantly With CD3, CDR4, and Foxp3. Correlation Coefficients Were Determined by Spearman Rank Correlation Analyses



therapy did not induce any significant change in the percentage of Tregs (Figure 6).

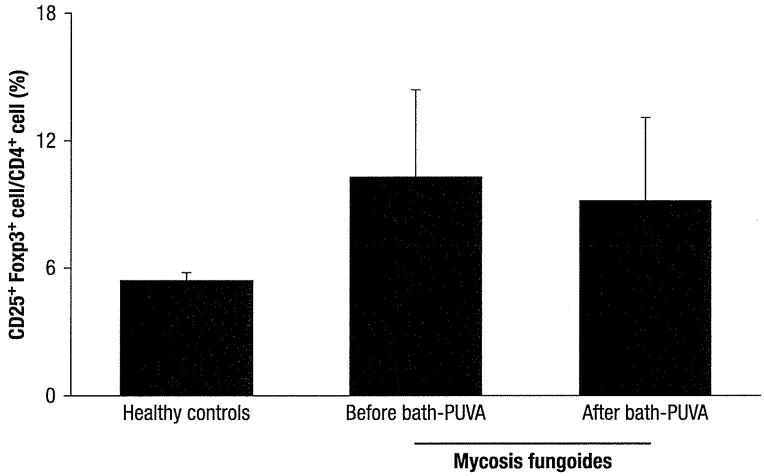
Discussion

The findings of the present study demonstrate that infiltration of both $CCR4^+$ and Tregs is eliminated by bath-PUVA therapy in patients with MF. The mechanism of the effectiveness of phototherapy for MF is not clear. For psoriasis treatment, there are 2 theories underlying the mechanisms of phototherapy: (1) induction of apoptosis in pathogenetically relevant cells[16] and (2) an immunosuppressive mechanism through the induction of Tregs.[7] The former is classified as an induction of apoptosis through oxygen radicals and caspase activation, leading to direct DNA damage. Photophoresis is a unique type of photochemotherapy using extracorporeal circulation and ultraviolet irradiation. In 1987, Edelson reported the efficacy of photophoresis treatment of cutaneous T-cell lymphoma.[17] A growing body of evidence indicates that photophoresis is also effective against other diseases.[18] Photophoresis induces monocytes to activate dendritic cells that express highly costimulatory molecules. The dendritic cells engulf the dying target cells,[19] leading to an immune response. Conversely, photophoresis induces antigen-specific Tregs. Therefore it is unclear how photophoresis regulates the immune responses toward both antiinflammatory and anticancer effects.

Leukocyte trafficking, which is critically regulated by chemokines and their receptors, shares many characteristics with tumor cell infiltration and metastasis. For example, CCR4 is a chemokine receptor selectively expressed on Tregs and Th2 cells[20-22] and also frequently expressed in ATLL cells, and its ligands TARC/CCL17 and macrophage-derived chemokine (MDC)/CCL22 are abundantly present in skin. We previously reported a significant association between the extent of CCR4 expression in ATLL cells and skin involvement.[9] With respect to MF, CCR4 expressed on the tumor cells also has a critical role in tumor formation in the skin.[23]

Generally, sIL2R is used as a tumor marker in cutaneous lymphoma such as in MF,[24] and in the present study sIL2R levels correlated positively with the number of infiltrating $CD3^+$ cells but not with that of infiltrating $CCR4^+$ and $Foxp3^+$ cells. TARC/CCL17 is also reported to be an MF tumor marker[15] and, as expected, serum TARC/CCL17 levels were significantly correlated with the numbers of infiltrating $CD3^+$, $CCR4^+$, and $Foxp3^+$ cells. These findings indicate that the number of the affected skin infiltrating $CD3^+$, $CCR4^+$, and $Foxp3^+$ cells reflect the disease activity of MF.

Figure 6 Fluorescence-Activated Cell Sorting (FACS) Analysis With Peripheral Blood Mononuclear Cells (PBMCs) Obtained From Healthy Volunteers or Patients With MF Before and After Bath–Psoralen Plus Ultraviolet A (PUVA) Therapy. There was No Significant Difference Between Patients With Mycosis Fungoides (MF) Before Bath-PUVA and Healthy Volunteers (Wilcoxon rank-sum test). Bath-PUVA Therapy Did Not Induce Any Significant Change in the Percentage of T-Regulatory Cells (Tregs). The Significance of Changes in the Variables Before and After Bath-PUVA Therapy was Examined Using the Wilcoxon Signed-Rank Test



PUVA is widely used as an effective treatment for cutaneous T-cell lymphoma.[25] MF and Sézary syndrome are the most frequent forms of cutaneous T-cell lymphoma. We previously reported that bath-PUVA therapy induces circulating Tregs in patients with psoriasis.[7] Tregs is a T-cell subset with immune function[26] that is associated with some immune diseases.[27] *FOXP3* is a master regulator gene for the differentiation of Tregs, and Foxp3 is a molecular marker of Tregs.[28] *FOXP3* gene transfection in naive T cells transforms naive T cells into Tregs and, simultaneously, naive T cells acquire CCR4 on their surface.[29]

In the present study, bath-PUVA therapy eliminated $Treg^+$ and $CCR4^+$ cells. Considering that there are some $CCR4^+$ cells among Tregs, the efficacy of bath-PUVA is similar to anti-CCR4 antibody treatment, as previously reported, regarding the decrease in the number of $CCR4^+$ cells.[8,12] In contrast, it was unclear that anti-CCR4 antibody was effective against $Foxp3^+$ cells. Based on the positive correlation between $CCR4^+$ and $CD3^+$cells after bath-PUVA therapy, the other type of T cells may have been eliminated.

Recently, Tregs were divided into naturally occurring Tregs and inducible Tregs.[30] Inducible Tregs are derived from the peripheral blood after antigen stimulation. In MF, Tregs are present in the initial stage, but the number of Tregs decreases in the more advanced stages.[31] This progression suggests a correlation between the number of Tregs and the prognosis of MF. Theoretically, the number of Tregs must be reduced in patients with MF. In psoriasis, bath-PUVA suppresses immunity and concomitantly induces improvement of the lesions. Immunosuppression might lead to an increase of the tumor cells in MF. In the present study, Tregs in the skin decreased after bath-PUVA. Moreover, the number of circulating Tregs in peripheral blood was not changed after bath-PUVA.

Based on these results, systemic immunosuppression is not induced by bath-PUVA therapy in patients with MF. It is generally accepted that increased Tregs in the tumor microenvironment have an important role in tumor escape from host immunity in several different types of cancer. Therefore depletion of Tregs in the tumor vicinity is considered a potential strategy for boosting antitumor immunity. In this context, the bath-PUVA therapy–induced reduction of Tregs observed in the present study may induce antitumor immunity and subsequent tumor elimination in MF skin lesions.

Clinical Practice Points

- MF is a malignant cutaneous lymphoma with a chronic disease progression.
- There are some clinical skin forms in MF according to STAGE. The tumor cells were T lymph cells, especially, it reported that CCR4 was expressed highly in the tumor cells.
- CCR4 is a chemokine receptor expressed on certain types of T cell neoplasms. In ATLL, the subsequent phase II clinical trials targeted CCR4 was started. For MF, some treatments were used such as topical steroid, phototherapy, and chemotherapy. Especially, bath-PUVA therapy was effective for the early STAGE of MF. However, the mechanism of the bath-PUVA therapy for MF was unclear.
- In the present study, bath-PUVA therapy decreased CCR4 positive cells and Treg in MF lesions, but did not induce circulating Treg, which might suppress effector T cells. Direct effects through skin lesions might eliminate both pathogenetically relevant cells and Treg.

- Considering that there are some CCR4-positive cells among Treg, the efficacy of bath-PUVA is similar to anti-CCR4 antibody treatment. Systemic immunosuppression was not induced by bath-PUVA therapy. It is generally accepted that increased Treg in the tumor microenvironment has an important role in tumor escape from host immunity in several different types of cancer.
- Based on these results, bath-PUVA therapy had possibility that widely applied for other disease.

Acknowledgments

We thank Kyowa Hakko, Kirin Co. (Tokyo, Japan) for providing the mouse anti-CCR4 monoclonal antibody (KM2160).

Disclosure

The authors have stated that they have no conflicts of interest.

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Cancer Science
The official journal of the Japanese Cancer Association JCA

Case Report

Stevens–Johnson Syndrome associated with mogamulizumab treatment of adult T-cell leukemia/lymphoma

Takashi Ishida,[1,6] Asahi Ito,[1] Fumihiko Sato,[2] Shigeru Kusumoto,[1] Shinsuke Iida,[1] Hiroshi Inagaki,[2] Akimichi Morita,[3] Shiro Akinaga[4] and Ryuzo Ueda[5]

Departments of [1]Medical Oncology and Immunology, [2]Anatomic Pathology and Molecular Diagnostics, [3]Geriatric and Environmental Dermatology, Nagoya City University Graduate School of Medical Sciences, Nagoya; [4]Kyowa Hakko Kirin, Tokyo; [5]Department of Tumor Immunology, Aichi Medical University School of Medicine, Nagakute, Japan

(Received December 25, 2012/Revised January 21, 2013/Accepted January 23, 2013/Accepted manuscript online January 30, 2013)

We report an adult T-cell leukemia/lymphoma patient suffering from Stevens–Johnson Syndrome (SJS) during mogamulizumab (humanized anti-CCR4 monoclonal antibody) treatment. There was a durable significant reduction of the $CD4^+CD25^{high}FOXP3^+$ regulatory T (Treg) cell subset in the patient's PBMC, and the affected inflamed skin almost completely lacked FOXP3-positive cells. This implies an association between reduction of the Treg subset by mogamulizimab and occurrence of SJS. The present case should contribute not only to our understanding of human pathology resulting from therapeutic depletion of Treg cells, but also alert us to the possibility of immune-related severe adverse events such as SJS when using mogamulizumab. We are currently conducting a clinical trial of mogamulizumab for CCR4-negative solid cancers (UMIN000010050), specifically aiming to deplete Treg cells. (*Cancer Sci* 2013; 104: 647–650)

Adult T-cell leukemia/lymphoma (ATL) is an aggressive peripheral T-cell neoplasm caused by HTLV-1. The disease is resistant to conventional chemotherapeutic agents, and has a very poor prognosis.[1] Mogamulizumab (KW-0761) is a defucosylated humanized monoclonal antibody targeting CC chemokine receptor 4 (CCR4).[2] A phase I clinical trial for relapsed CCR4-positive peripheral T-cell neoplasms, including ATL, and a phase II study for relapsed ATL have been conducted with mogamulizumab.[3,4] This agent was subsequently approved for the treatment of relapsed or refractory ATL in Japan, the first country in the world to do so, in March 2012. Mogamulizimab went on sale on 29 May 2012. The interim report for the post-marketing surveillance from 29 May to 28 September 2012 revealed skin-related severe adverse events (SAE), as defined by the Medical Dictionary for Regulatory Activities Terminology/Japan, in nine patients. Thus, during only the first 4 months of use, 9 skin-related SAE, including 4 cases of Stevens–Johnson Syndrome (SJS)/toxic epidermal necrolysis (TEN) were reported, with 1 SJS/TEN fatality. These skin-related, potentially fatal SAE are certainly a challenge to the free use of this agent and clearly require investigation. Therefore, here we report an informative ATL patient suffering from SJS on mogamulizumab treatment, focusing on the reduction of the regulatory T (Treg) cell subset ($CD4^+CD25^{high}FOXP3^+$) caused by the antibody.

Case Report

A 71-year old woman was admitted due to elevation of her lymphocyte count. She had been diagnosed as suffering from acute-type ATL nearly 5 months prior to admission. She had received VCAP-AMP-VECP chemotherapy[5] followed by oral sobuzoxane in another hospital, and achieved a transient partial remission. We started mogamulizumab to treat the flare-up of ATL disease (Fig. 1). Grade 1 skin eruptions appeared around her neck after three antibody infusions. Because we were also giving her antibacterial (ciprofloxacin hydrochloride), fungal (itraconazole), pneumocystic (sulfamethoxazole-trimethoprim) and viral (aciclovir) prophylaxes in addition to stomach medicine (lansoprazole), we judged the skin event to be due to drug eruption caused by one of these concomitant drugs. Therefore, we stopped all five, but continued with mogamulizumab. Despite their discontinuation and treatment with topical steroids, the skin rashes continued to worsen. We started the patient on 30 mg oral prednisolone, which improved the skin symptoms. The patient was then able to complete the eight planned infusions, and oral prednisolone was tapered off. She was discharged from hospital 8 days after her eighth infusion (day 65), and thereafter seen as an outpatient. However, she had to be readmitted as an emergency patient at day 75 because of fulminant skin rashes. These included erythemas, scale-like plaques, vesicles, blisters and erosions over many areas of the body. Her lips were swollen and oral mucosa was erosive (Fig. 2a). Skin biopsy revealed marked liquefaction, degeneration and perivascular inflammation with dominant CD8-positive cells but almost complete lack of FOXP3-positive cells (Fig. 2b). We diagnosed her as a SJS, and immediately started steroid pulse therapy (methylprednisolone 500 mg/day ×3 days), followed by oral prednisolone. Her skin and mucosal lesions improved gradually, and became inactive. At the same time, her general condition improved. Thus, we again tapered the steroid dose, and she was discharged at day 144. However, she had to come back yet again as an emergency patient on day 151 for the same reason as before, with fulminant skin rashes. We prescribed her mini-steroid pulse therapy (methylprednisolone 125 mg/day ×1 day), followed by oral prednisolone. Once more, her skin lesions improved gradually. Over this whole period, complete ATL remission was maintained by mogamulizumab. The HTLV-1 provirus load in PBMC pre-treatment, and at days 121 and 162 was 750.1, 0.0 (under the limit of detection) and 0.8 copies/1000 cells, respectively. These post-treatment values are strikingly low, considering that median HTLV-1

[6]To whom correspondence should be addressed.
E-mail: itakashi@med.nagoya-cu.ac.jp

doi: 10.1111/cas.12116

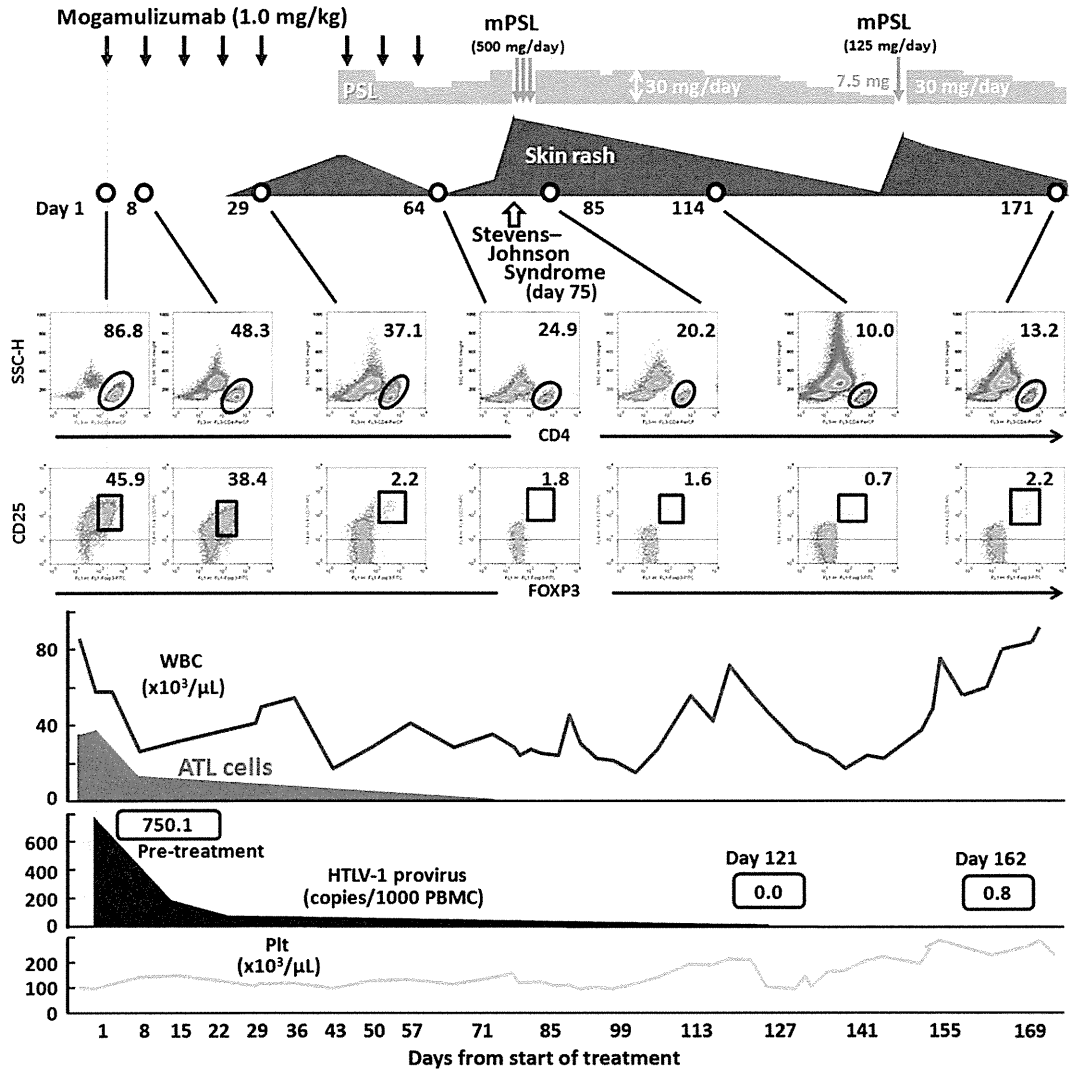

Fig. 1. Clinical course of an ATL patient receiving mogamulizumab monotherapy. ATL; adult T-cell leukemia/lymphoma; mPSL, methylprednisolone; Plt, platelet PSL; prednisolone; WBC, white blood cell.

load in asymptomatic carriers reported by other investigators is 18.0 copies/1000 cells.[6]

We also analyzed CD4, CD25 and FOXP3 expression by PBMC during and after antibody treatment (Fig. 1, middle panels). Before treatment, the majority of the patient's PBMC consisted of CD4-positive and CD25-positive ATL cells. Just before the 5th antibody infusion (day 29), around the time when her skin rash first appeared, the proportion of $CD25^{high}$-$FOXP3^{+}/CD4^{+}$ cells was markedly reduced, to 2.2%. This is low even compared to healthy individuals ($CD25^{high}$-$FOXP3^{+}/CD4^{+}$ cells, mean 3.3%, median 3.3%, range 2.6–4.4%) (Fig. 3). Around the time of SJS onset, the proportion of cells in the Treg subset was further reduced. The proportion of $CD25^{high}FOXP3^{+}/CD4^{+}$ cells at days 64, 85 and 114 was 1.8%, 1.6% and 0.7%, respectively. The striking reduction of the Treg subset persisted until 4 months after the last of the eight antibody infusions (day 171).

Discussion

Drugs often induce adverse cutaneous reactions of varying severity, ranging from simple uncomplicated eruptions to potentially fatal eruptions, such as SJS and TEN, within the spectrum of severe adverse reactions affecting skin and mucosa. Although many factors that might cause variability in the clinical course of such adverse reactions have been suggested, it remains unknown which factors are predominantly involved in these processes. The most prevalent severe drug eruptions are thought to be mediated by drug-reactive T-cells,[7] although we also need to be aware of the alternative view that severe drug eruptions are due to a dysregulated immune system. In this regard, an effect mediated by Treg cells is a likely candidate in severe drug eruptions. Indeed, it is reported that Treg cells can prevent experimentally-induced epidermal injury mimicking TEN in an animal model.[8] Furthermore, Takahashi *et al.* (2009) report that Treg cell function is profoundly impaired in patients with TEN.[9] Consistent with these reports, a marked reduction of the Treg subset was observed in the present case.

Mogamulizumab is the first therapeutic agent targeting CCR4, which is expressed on Treg cells,[10,11] to receive marketing approval anywhere in the world. The reduction of the Treg subset seen here was not specific to the present case, but is commonly observed in ATL patients receiving mogamulizumab. In fact, skin rashes were observed as a frequent non-hematologic adverse event (AE) (63%), mostly occurring

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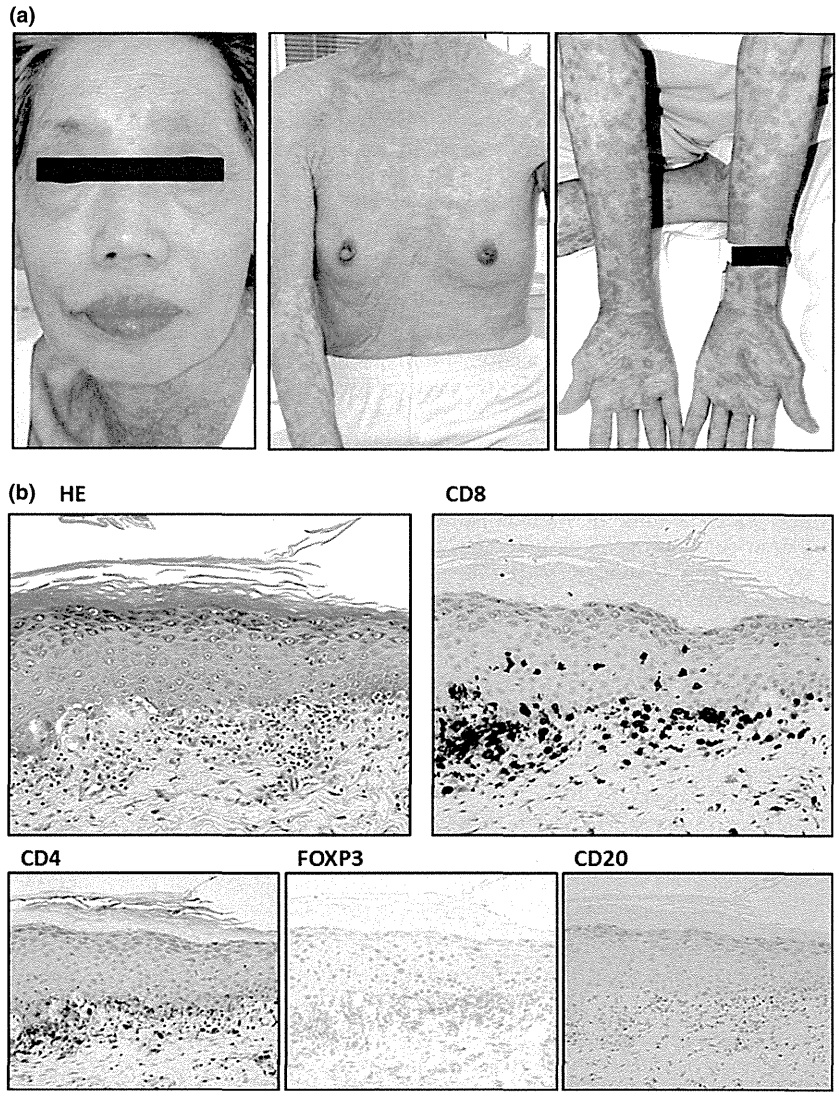
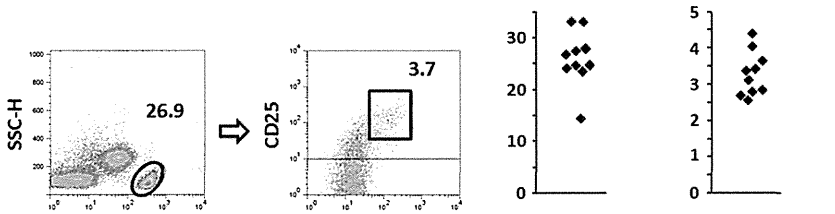


Fig. 2. (a) Macroscopic observations of the patient's skin on the day she was diagnosed with Stevens–Johnson Syndrome. (b) Corresponding skin biopsy showing liquefaction, degeneration and perivascular inflammation with dominant CD8-positive cells but almost no FOXP3-positive cells.



	$CD4^+$/PBMC (%)	$CD25^{high}$ $FOXP3^+$/CD4 + cells (%)
Mean	26.1	3.3
Median	25.9	3.3
Range	14.5–33.3	2.6–4.4

Fig. 3. $CD4^+CD25^{high}FOXP3^+$ regulatory T cells in PBMC from healthy volunteers (n = 10).

after the fourth or subsequent infusions in the phase II study.[4] The present case was one of these patients. It has been reported that alterations in $CD4^+CD25^+FOXP3^+$ Treg cell frequencies and/or function may contribute to various types of autoimmune diseases.[12] Because the CCR4 molecule aids lymphocyte skin-specific homing,[13] it is not unexpected that skin rashes, which could be an immune-related AE, will be frequently observed in ATL patients receiving mogamulizumab. Because it is an urgent issue to identify which factors determine the severity of immune-related skin disorders associated with mogamulizumab treatment, further investigation on this matter are clearly warranted.

However, reduction of Treg cells is a promising strategy for boosting antitumor immunity in cancer patients, because these cells are increased in the tumor microenvironment and may play an important role in tumor escape from host immunity in several different types of cancer.[14,15] Thus, reduction of Treg cells by mogamulizumab in cancer patients would have both potential benefits leading to enhanced antitumor immunity, but also pose risks of autoimmune disease. The skin-related SAE, including SJS/TEN, are representative of the latter. Currently, several clinical trials of mogamulizumab are being conducted worldwide, not only for ATL, but also other types of lymphoma. In addition, we are currently conducting a clinical trial of mogamulizumab for CCR4-negative solid cancers (UMIN000010050), specifically aiming to deplete Treg cells. Therefore, it is a matter of some urgency to establish the safest and most effective treatment strategies for using mogamulizumab not only in ATL patients but also other types of cancer, to maximize benefit and minimize risk.

In summary, the present case should contribute not only to our understanding of human pathology resulting from therapeutic depletion of Treg cells, but also alert us to the possibility of immune-related SAE, such as SJS/TEN, when using mogamulizumab.

Acknowledgments

The authors thank the husband of the patient for consenting to the publication of her clinical details. The present study was supported by Grants-in-Aid for Young Scientists (A) (No. 22689029), Scientific Research (B) (No. 22300333), and Scientific Support Programs for Cancer Research (No. 221S0001) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, a Grant-in-Aid from the National Cancer Center Research and Development Fund (No. 23-A-17), and Health and Labour Sciences Research Grants (H22-Clinical Cancer Research-general-028 and H23-Third Term Comprehensive Control Research for Cancer-general-011) from the Ministry of Health, Labour and Welfare, Japan.

Disclosure Statement

Nagoya City University Graduate School of Medical Sciences has received research grant support from Kyowa Hakko Kirin for works provided by Takashi Ishida. Takashi Ishida received honoraria from Kyowa Hakko Kirin for his works. Shiro Akinaga is an employee of Kyowa Hakko Kirin. No other conflict of interest relevant to this article is reported.

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doi: 10.1111/cas.12116

J Gastroenterol (2013) 48:1373–1383
DOI 10.1007/s00535-013-0763-8

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Which patients respond best to hepatitis B vaccination after a hepatitis B virus-related liver transplantation?

Akinobu Takaki · Takahito Yagi · Tetsuya Yasunaka · Hiroshi Sadamori · Susumu Shinoura · Yuzo Umeda · Ryuichi Yoshida · Daisuke Sato · Daisuke Nobuoka · Masashi Utsumi · Yuko Yasuda · Eiichi Nakayama · Yasuhiro Miyake · Fusao Ikeda · Hidenori Shiraha · Kazuhiro Nouso · Toshiyoshi Fujiwara · Kazuhide Yamamoto

Received: 31 August 2012 / Accepted: 31 January 2013 / Published online: 23 February 2013

Abstract
Background A combination of hepatitis B immunoglobulin and nucleos(t)ide analogues is the current standard of care for controlling hepatitis B recurrence after orthotopic liver transplantation (OLT). However, frequent immunoglobulin treatment is expensive and inconvenient. This study investigated the efficacy of hepatitis B virus (HBV) vaccination in preventing the recurrence of hepatitis B after living donor OLT.
Methods Twenty-seven patients who had undergone living donor OLT participated in the study; five had acute HBV infected liver failure (ALF-OLT) and 22 had HBV related liver cirrhosis (LC-OLT). Hepatitis B surface antigen (HBsAg)-containing vaccine was administered to them for at least 1 year after transplantation and continued once monthly for up to 36 months post-OLT. Patients who had anti-HBs antibody titers above 100 mIU/mL for a minimum of 6 months without immunoglobulin administration were defined as good responders; the others were defined as poor responders. Interferon-γ enzyme-linked immunospot assays against HBs and HBc antigens were used to assay cellular immune responses.
Results All five of the ALF-OLT patients had good responses after a median of four (range 2.5–5) vaccinations. Nine of the 22 LC-OLT patients had good responses after a median of 19 (range 11.5–30) vaccinations. Among the LC-OLT group, those with livers donated by relatively higher-aged, marital and high-titer anti-HBs antibody donors were good responders. LC-OLT patients classed as good responders showed interferon-γ responses comparable to those of the ALF-OLT patients.
Conclusions The ALF-OLT and LC-OLT patients who received livers from relatively higher-aged, marital, high-titer anti-HBs antibody donors were the best candidates for HBV vaccine administration. Boosting donors before transplantation may facilitate later vaccine response of the recipients.

Keywords Vaccination · Living donor liver transplantation · Hepatitis B immunoglobulin · Marital donor · Immune response

A. Takaki (✉) · T. Yasunaka · Y. Miyake · F. Ikeda · H. Shiraha · K. Nouso · K. Yamamoto
Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan
e-mail: akitaka@md.okayama-u.ac.jp

T. Yagi · H. Sadamori · S. Shinoura · Y. Umeda · R. Yoshida · D. Sato · D. Nobuoka · M. Utsumi · T. Fujiwara
Department of Gastroenterological Surgery Transplant and Surgical Oncology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan

Y. Yasuda
Okayama University Hospital, 2-5-1 Shikata-cho, Kita-Ku, Okayama 700-8558, Japan

E. Nakayama
Kawasaki University of Medical Welfare, 288 Matsushima, Kurashiki, Okayama 701-0193, Japan

Introduction

Prior to the introduction of effective post-transplantation antiviral prophylaxis, liver transplantation for hepatitis B virus (HBV)-related disease was usually followed by immediate HBV reinfection of the allograft, resulting in a fatal hepatitis B recurrence [1–3]. Recent studies have found that treatment with a combination of hepatitis B

immunoglobulin (HBIg) and nucleos(t)ide analogues decreases the risk of hepatitis B recurrence, and achieves a higher rate of graft survival [4–8]. However, long-term administration of HBIg is associated with several unresolved issues, including limited availability and extremely high cost, so several protocols for treatment with low-dose HBIg in combination with nucleos(t)ide analogue have been reported [9–12]. Previously, we reported that treatment with high-dose HBIg in the early period post-transplantation followed by low-dose HBIg with nucleos(t)ide analogues offers reliable, cost-effective control of hepatitis B recurrence [13]. However, even with such a simplified protocol, patients would still need to receive a drip infusion or intramuscular injection of hundreds to thousands of units of HBIg every 2–3 months.

Active immunization of post-orthotopic liver transplantation (OLT) recipients with HBV vaccine is a recently emerging approach. However, most studies report low response rates, even with double concentration of vaccines or prolonged vaccination regimens [14, 15]. Patients who had not been HBV carriers [e.g., acute liver failure (ALF) patients following sexual transmission of HBV as an adult; or non-chronic HBV carrier patients who received hepatitis B core antibody (HBcAb)-positive livers] are accepted as good candidates for vaccine administration [15, 16]. Vaccination in patients who have been HBV carriers or liver cirrhosis (LC) patients typically yields disappointing results [14, 15]. Understanding how different cohorts respond to HBV vaccination is critical to the design of safe, cost-saving, and custom-designed prophylaxis protocols.

It remains unclear to what extent cellular immune responses may contribute to protection from HBV reinfection. Since non-carrier patients respond well to the HBV vaccination, immune tolerance is expected to play a large role in this process. Yet only a few reports have mentioned T cell immune reaction after HBV-related OLT [14].

In this report, we assessed a monthly, long-term vaccination protocol starting 1 year after OLT, to investigate those characteristics that could discriminate between the vaccine-responsive and non-responsive patients. In addition to anti-hepatitis B surface (anti-HBs) antibody titer due to a humoral immune response, CD4 T cell immune responses to hepatitis B surface antigen (HBsAg) were used to assess the cellular immune response to vaccination in immunocompetent patients.

Methods

Patients

From October 1996 to June 2011, OLT was performed in 264 adults at Okayama University Hospital. Of these, ten patients had ALF due to acute HBV infection. Thirty-seven patients had end-stage LC due to chronic life-long HBV infection. Five-year survival rates were 88 and 87 % for HBV-related ALF patients and for HBV-related LC patients, respectively.

The HBV vaccine was administered to five ALF patients (ALF-OLT) and 22 LC patients (LC-OLT). The general characteristics of the patients included in this study are summarized in Table 1. All of them received living donor liver transplantation (LDLT). The numerical data are expressed as median and interquartile range values, and categorical data are presented as positive counts or percentages in all tables.

For analysis of the HBV-specific cellular immune response (Table 2), the study enrolled all five ALF-OLT patients, along with 15 of the 22 LC-OLT patients. Additionally, 11 healthy volunteers who had received the HBV vaccine and developed a successful anti-HBs antibody response (termed 'Healthy vaccine'), ten patients with chronic hepatitis B (termed 'Chronic hepatitis'), and five patients who recovered from acute hepatitis B (termed 'Self-limited') were enrolled as controls. The five patients who recovered from acute hepatitis B had a history of acute hepatitis B diagnosed with high-titer IgM-HBc antibody response, and presented as HBsAg negative, anti-HBs antibody positive, anti-HBc antibody positive at the time of

Table 1 Patient characteristics

N	ALF 5	LC 22
Recipient related factors		
Age at OLT	29 (27–46)	53 (47–56)
Age at start of vaccine	36 (30–51)	56 (49–59)
Sex (M)	1 (20 %)	19 (86 %)
HBsAg at OLT	0.7 (0–1)	2000 (100–2000)
HBV DNA at OLT (≥3.7)	0 (0 %)	8 (36 %)
MELD at OLT	21 [19–21]	15 [9–18]
HCC at OLT (+)	0 (0 %)	15 (68 %)
Donor related factors		
Age at OLT	32 (27–44)	46 (31–49)
Sex (M)	4 (80 %)	9 (40 %)
ABO (identical)	4 (80 %)	12 (54 %)
Blood relation (no)	0 (0 %)	8 (36 %)
Anti-HBs antibody (>100)	1 (20 %)	9 (40 %)
Anti-HBc antibody (+)	1 (20 %)	11 (50 %)
Anti-HBc(+)/anti-HBs(+)	1 (20 %)	10 (45 %)
Anti-HBc(+)/anti-HBs(−)	0 (0 %)	1 (4 %)
Anti-HBc(−)/anti-HBs(+)	0 (0 %)	0 (0 %)

ALF acute liver failure, *LC* liver cirrhosis, *OLT* orthotopic liver transplantation, *MELD* Model for End-stage Liver Disease, *HCC* hepatocellular carcinoma

Table 2 Characteristics of the cases for HBV antigen-specific T cell response

N	Healthy vaccine	Chronic hepatitis	Self-limited	ALF-OLT	LC-OLT-good	LC-OLT-poor
	11	10	5	4	8	7
Age	29 (28–31)	53 (42.5–61)	67 (58.5–77)	41.5 (37.2–47.2)	60 (53–62)	55 (40–58)
Sex [M (%)]	10 (91)	7 (70)	2 (40)	0 (0)	8 (100)	7 (100)
HBs Ag (+)	0	10 [titer 2000 (1893–2000)]	0	0	0	0
HBs Ab (IU/l) (>100/≤100)	8/3	0/10	2/3	2/2	4/4	1/6

LC-OLT-poor patients received HBIG within 3 months

Age and HBsAg were shown as median (interquartile range)

ALF-OLT acute liver failure patients who received OLT, *LC-OLT-good* liver cirhosis patients who received OLT and had a good vaccine response, *LC-OLT-poor* liver cirrhosis patients who received OLT and had a poor vaccine response

the study. The chronic hepatitis B patients were followed for several years at our hospital and all were HBsAg positive with a median HBV-DNA titer of 2.5 (interquartile range 2.1–4.2) logcopies/mL. The healthy volunteers had no HBsAg and anti-HBc antibodies, and the median anti-HBs antibody level was 240 (interquartile range 100–797) mIU/mL.

Informed consent was obtained from each patient included in the study, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in the approval by the Ethics Committee at the Okayama University Hospital.

Antiviral prophylaxis

Our HBV prophylaxis protocol was as follows. We administered HBIg at 200 IU/kg intraoperatively. Recipients were administered another 2000 IU/week HBIg for an additional 1 week post-operatively. HBIg (2000 IU) was administered thereafter only when anti-HBs antibody titers fell below 100 mIU/mL. After 6 months, HBIg was administered only to maintain anti-HBs antibody titers at >10 mIU/mL. We measured levels of HBsAg and anti-HBs antibody and/or HBV-DNA every month for 6 months after LDLT, and every 2–3 months thereafter. Three of the ALF-OLT patients were anti-HBs antibody positive at the time of OLT, these patients were not administered nucleos(t)ide analogues. The remaining two ALF-OLT patients, and all of the LC patients were given nucleos(t)ide analogues. The two ALF-OLT patients were given lamivudine (LAM), and of the 22 LC-OLT patients, 14 received LAM, six were given LAM + adefovir dipivoxyl (ADV), and two received entecavir (ETV). Administration of nucleos(t)ide analogues was started a minimum of 1 month pre-operatively, when possible.

Post-OLT re-activation of HBV was defined as continuous positivity for serum HBsAg and/or serum HBV-DNA.

HBV vaccine protocol

HBV vaccine administration was initiated at least 1 year after OLT, and when patients showed no active infection or rejection episode in the preceding month. The vaccine consisted of recombinant purified HBsAg (Bimmugen; Kaketsuken, Kumamoto, Japan). Ten micrograms were administered every 1–2 months. Based on the effect of the vaccine, patients were classified as "good responders; LC-OLT good" or "poor responders; LC-OLT poor". Patients who showed anti-HBs antibody titers above 100 mIU/mL without HBIg for a minimum of 6 months were defined as good responders, since all of these patients did not need HBIg administration for an additional 2 years (median) of follow-up. All other patients were defined as poor responders. Patients who showed a good response within 36 months were given additional vaccinations when their anti-HBs antibody titer decreased, whereas vaccination was stopped in patients who showed no good response after 36 months.

Immune suppression

Patients were treated using a standard immunosuppressive regimen (tacrolimus or cyclosporine A with steroids and/or mycophenolate mofetil). One patient was free from calcineurin inhibitors at the time of vaccine administration.

Routine laboratory tests and serum HBV-DNA assay

Hepatitis B surface antigen, anti-HBs antibody, hepatitis Be antigen (HBeAg), and anti-HBe antibody (HBeAb) levels were measured routinely using a commercially available chemiluminescent enzyme immunoassay system (Lumipulse System; Fujirebio, Tokyo, Japan). HBV-DNA levels were measured using a transcription-mediated amplification assay (TMA) (SRL, Tokyo, Japan), a polymerase chain reaction (PCR) assay (Amplicor HBV

Monitor assay; Roche Diagnostics, Tokyo, Japan), or a real-time PCR assay (COBAS TaqMan HBV Test; Roche Diagnostics).

HBV recombinant proteins for cellular immune response analysis

Hepatitis B virus recombinant protein HBsAg was purchased from Advanced ImmunoChemical, Inc. (Long Beach, CA). Recombinant protein hepatitis B core antigen (HBcAg) was purchased from the Institute of Immunology (Tokyo, Japan). These proteins were used as stimulating antigens at 1 μg/mL for the enzyme-linked immunospot (ELISPOT) assay.

CD14-positive monocyte isolation and myeloid DC generation

Mononuclear cells were separated from peripheral blood by centrifugation on the Ficoll-Hypaque density gradient (Amersham Pharmacia, Uppsala, Sweden), as previously described. CD14-positive monocytes were purified using microbeads (Miltenyi Biotec, Auburn, CA) in accordance with the protocols of the manufacturer. Subsequently, CD4-positive T cells (T4) were positively sorted in the same way. T4 cells were frozen immediately. CD14-positive cells were cultured at 1×10^6/mL in RPMI containing 5 % heat-inactivated human AB serum (ICN Biomedicals; Aurora, OH) supplemented with 100 ng/mL of granulocyte macrophage colony-stimulating factor (kindly provided by Kirin Pharma, Tokyo, Japan) and 50 ng/mL of interleukin-4 (kindly provided by Ono Pharmaceuticals, Osaka, Japan) at 37 °C in 5 % CO_2 for 5 days. Cells were confirmed to be CD11c-positive myeloid immature dendritic cells (DC).

Interferon-γ (IFNγ) ELISPOT assay with myeloid DC and CD4-positive T-cells

The immature DC cultures were exposed to recombinant HBsAg and HBcAg (1 μg/mL each) for 1 day. To mature the DCs, 1 ng/mL of lipopolysaccharide (LPS) (Sigma, St. Louis, MO) was added to the culture 1 day after HBV protein addition. On the same day, mouse anti-human interferon-γ antibody (MABTECH, Sweden) was diluted to 5 μg/mL with ELISPOT buffer (0.159 % Na_2CO_3, 0.293 % $NaHCO_3$) and coated overnight at 4 °C onto 96-well filtration plates (Millipore, Billerica, MA) at 100 μL per well. The coated plate was washed with phosphate-buffered saline (PBS) and blocked with 10 % fetal calf serum in RPMI1640 medium for 1–2 h. Myeloid DCs were counted and seeded at 5×10^3/well. Cryopreserved T4 cells were thawed, counted, and seeded at 2×10^5/well. On the next day, the plate was washed six times with PBS. Wells were coated with rabbit anti-interferon-γ serum (diluted to 1/800 in PBS), and the plate was incubated at 37 °C for 2 h. The plate was washed six times with PBS and coated with goat anti-rabbit immunoglobulin G-alkaline phosphatase (IgG-AP; Southern Biotech, Birmingham, AL) diluted to 1/2000 with PBS. After a 1 h incubation at 37 °C, the plate was washed six times with water and spots were developed using 5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt and nitroblue tetrazolium chloride (BCIP/NBT) as a substrate. Spot development was stopped after 10 min by washing with distilled water. The spots were viewed and counted under a microscope.

Statistical analysis

Statistical comparisons were performed using JMP version 9 (SAS Institute, Cary, NC, USA). The Wilcoxon rank-sum test was used to compare the continuous data and the Chi-square test was used to compare categorical data. For multivariate analysis, logistic regression analysis was used. The Steel–Dwass test was used for multiple group analysis. A p value of <0.05 was considered significant.

Results

The effects of HBV vaccination

None of the patients in the ALF-OLT group showed reactivation of the virus. One patient of the LC-OLT group showed transient positive responses for HBsAg and HBV DNA, however, these became negative again with frequent HBIg administration. At the final observation point, no patients showed HBsAg or HBV DNA-positive response. All five ALF-OLT patients had good responses to vaccination (Table 3). A median of four (range 2.5–5) vaccinations were sufficient to induce a good response. In contrast, LC-OLT patients were less responsive, with only nine of 22 displaying a good response. Additionally, these nine good responders required a median of 19 (range 11.5–30) vaccinations before these patients could be weaned from HBIg administration (Fig. 1).

Table 3 Results of HBV vaccination

N	ALF 5	LC 22
Response to vaccination (good/poor responders)	5/0	9/13
Number of vaccinations require before ceasing HBIg treatment	4 (2.5–5)	19 (11.5–30)

HBIg Hepatitis B immunoglobulin

Vaccine safety

None of the patients showed any adverse reactions as judged by their general condition, or by laboratory examination. One patient reported itchiness after injection of the eighth vaccination dose, although the symptom subsequently stopped.

The characteristics of vaccine responsiveness in LC-OLT patients

To determine the characteristics for defining a good response in LC-OLT patients, clinical data from recipients and donors were investigated (Table 4). The background data of the recipients, including HBV-DNA levels, HBeAg positive reactions, HBsAg levels at the time of OLT, and the anti-HBs antibody titer at the time of the initial vaccination did not differ between the good and poor responder groups (Table 5). However, the donor-related factors did differ. Notably, the good responders' donors were relatively high in age ($p = 0.019$) and not blood relatives of the recipients ($p < 0.001$). These donors (to good responders) showed high anti-HBs antibody titers at the time of OLT ($p = 0.038$). Since all of the patients in this study received LDLT, non-blood-related donors all corresponded to spouses of the OLT recipients. Multivariate logistic regression analysis was carried out with the following variables: donor age at OLT $\geq$47, non-blood-related donor, donor anti-HBs antibody titer >100 mIU/mL (Table 6). A status of non-blood-related donor was identified as a significant independent predictor of a good response to vaccination. Since the donor anti-HBs antibody was one of the factors associated with a good response, we asked whether the donors had received vaccination, and found that none of them had ever received an HBV vaccine. As shown in Table 4, none of the donors showed the anti-HBc antibody-negative, anti-HBs antibody-positive condition which indicates vaccine-induced seropositivity to the HBs antigen.

HBV antigen-specific immune responses

To determine the effectiveness of vaccine-induced cellular immune responses in post-OLT patients, we used the IFN-γ ELISPOT assay. First of all, we analyzed the clinical characteristics of those patients showing strong HBsAg-specific T cell immune responses when compared with those of non-transplanted patients, and vaccine-induced anti-HBs antibody-positive, healthy volunteers (Fig. 2). The patients with stronger HBsAg-specific CD4 T cell IFN-γ responses (equal or more than the median; 7 spots) showed lower levels of HBV DNA, lower HBsAg, higher anti-HBs antibody titer, and higher HBcAg-specific immune responses. The HBsAg and HBcAg-specific CD4 T cell immune response under different clinical conditions is shown (Fig. 3). Volunteer controls who were positive for anti-HBs antibodies (as a result of previous vaccine administration) showed numerous HBsAg-specific IFNγ spots. Spot numbers were reduced in control chronic hepatitis B patients, but remained high (against both HBsAg and HBcAg) in acute resolved hepatitis B patients. The ALF-OLT and LC-OLT good responders had relatively higher HBsAg-specific T-cell immune responses than LC-OLT poor responders. The LC-OLT patients with successful vaccine-induced humoral immune responses also showed higher cellular immune responses than control chronic hepatitis B patients. The LC-OLT patients with poor vaccine responses also had low cellular responses, similar to those seen in chronic hepatitis B patients.

Discussion

In this study we found that HBV vaccination was effective in OLT patients whose donors were relatively high in age, marital (non-blood-related), with high-titer anti-HBs antibodies. The multivariate analysis revealed that a marital (non-blood-related) donor was the only factor that associated strongly with a good response to vaccine. Among these OLT recipients, a good response to vaccination included effective responses in both the humoral and cellular arms of the immune system.

Controlling HBV reactivation after OLT is critical. In the absence of prophylaxis, hepatitis B recurs very frequently and results in early graft failure. The prophylaxis protocols have progressed from HBIg immunoprophylaxis in the early 1990s, to lamivudine in the late 1990s, to the more recent application of HBIg combined with nucleos(t)ide analogues. In 1991, Muller et al. [17] reported the first use of long-term HBIg immunoprophylaxis, reducing the HBV recurrence rate to 25 % after 6 months of OLT and 18 % after 12 months. A multicenter study revealed that the three-year risk of HBV recurrence was 75 ± 6 % without HBIg, 74 ± 5 % with short-term (2-month) HBIg, and 36 ± 4 % with long-term (>6-month) HBIg treatment [18]. Patients who were positive for HBeAg or HBV-DNA displayed the greatest risk of recurrence (83 %); patients with acute fulminant liver failure showed the lowest risk (16 %).

In 1996, Grellier et al. [19] reported a trial of LAM as a prophylactic treatment, achieving 18 % recurrence of HBV at 6 months after OLT. However, the long-term recurrence rate at 3 years after OLT progressed to 41 %, indicating that LAM monotherapy is not recommendable for post-transplantation prophylaxis.

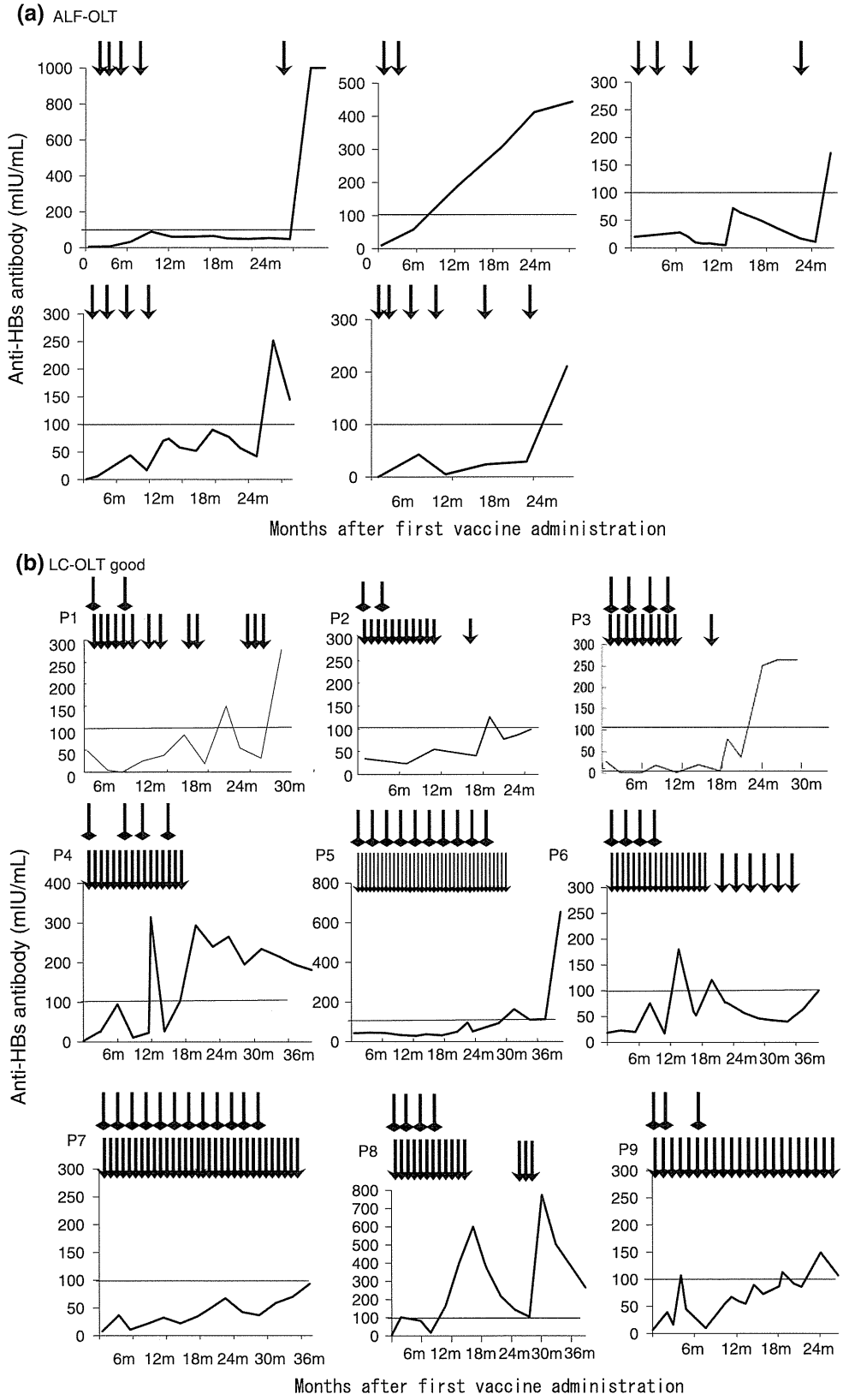

Fig. 1 Individual patients' timecourse of anti-HBs antibody titer after vaccine administration. The timecourse of the anti-HBs antibody titer after the first vaccine administration is shown. The *arrowhead* indicates a vaccine administration point, and the *square head* indicates an HBIg administration point. **a** Patients who received orthotopic liver transplantation (OLT) due to hepatitis B-related acute liver failure (ALF-OLT). All patients had a good response to vaccination. **b** Patients who received OLT due to liver cirrhosis with a good response to vaccination (LC-OLT good). **c** LC-OLT patients with a poor response to vaccination (LC-OLT poor)

Although monotherapy with HBIg or LAM resulted in a high rate of recurrence, a combination of these agents has been administered with reasonable success. In 1998, Markowitz et al. [20] reported no recurrences after 1 year of combination therapy. Since HBIg is very expensive, several reports have described modified combination

Fig. 1 continued

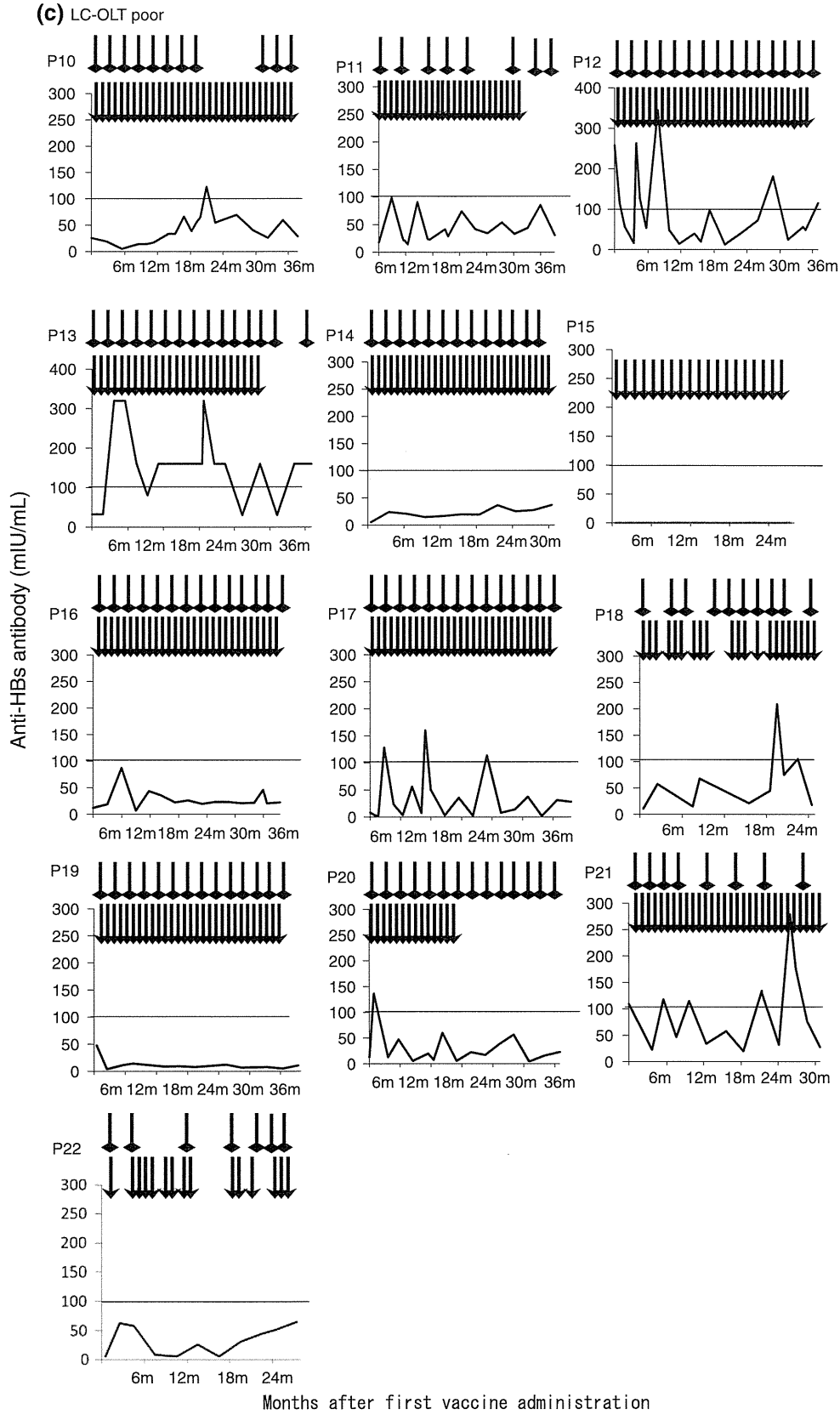

Table 4 LC patient characteristics

Characteristics of recipients												Characteristics of donors						
Patient's number	Response to vaccine	Age (year) at OLT	Sex	HBsAg (mIU/mL) at OLT	HBsAb at OLT	HBeAg/HBeAb at OLT	HBV DNA (logcopies/mL) at OLT	MELD at OLT	HCC at OLT	Time of vaccination (months post-OLT)	HBsAb (mIU/mL) at vaccine	NA at vaccine	Age at OLT	Sex	Blood relation	ABO compatibility	HBcAb	HBsAb	HBsAb (mIU/mL)
1	Good	56	M	100	–	–/+	<3.7	17	+	51	49	LAM	52	F	–	Compatible	–	–	<0.1
2	Good	48	M	>2000	–	+/+	3.5	20	+	24	23	LAM	46	F	–	Compatible	+	+	134
3	Good	44	M	100	–	+/–	<3.7	12	–	55	1	LAM	48	F	+	Identical	+	+	189
4	Good	50	M	>2000	–	+/–	3.4	9	+	42	25	LAM + ADV	48	F	–	Compatible	+	+	627
5	Good	54	M	>2000	–	–/+	3.8	15	–	40	43	LAM + ADV	48	F	–	Compatible	–	–	<0.1
6	Good	57	M	>2000	–	–/+	2.7	15	+	45	18	LAM	53	F	–	Identical	–	–	<0.1
7	Good	48	M	642	–	+/–	4.8	17	–	29	7	LAM	44	F	–	Compatible	+	+	179
8	Good	47	F	>2000	–	+/–	4.5	12	–	19	6	LAM	50	M	–	Compatible	+	+	1000
9	Good	55	M	>2000	–	+/–	6.1	21	+	49	6	LAM + ADV	48	M	+	Identical	+	+	133
10	Poor	52	M	>2000	–	+/–	5.3	8	+	25	4	LAM	21	M	+	Compatible	+	+	1000
11	Poor	62	M	>2000	–	–/+	<2.6	8	+	13	17	LAM + ADV	36	M	+	Identical	–	–	<0.1
12	Poor	39	M	>2000	–	+/–	<2.6	7	–	30	169	LAM	35	F	+	Identical	–	–	<0.1
13	Poor	49	M	100	–	–/+	4.0	21	+	107	32	LAM	22	F	+	Identical	–	–	<0.1
14	Poor	26	M	100	–	+/–	5.5	20	+	75	30	LAM	53	M	+	Identical	+	+	397
15	Poor	54	F	100	–	+/–	4.6	22	+	55	1	LAM	28	M	+	Identical	–	–	<0.1
16	Poor	50	M	160	–	–/+	2.7	18	+	38	6	LAM	25	M	+	Compatible	+	–	<0.1
17	Poor	44	M	>2000	–	–/+	<2.6	15	–	32	14	LAM	47	F	+	Compatible	–	–	<0.1
18	Poor	55	F	>2000	–	+/–	2.8	10	+	19	10	LAM + ADV	51	F	+	Identical	+	+	44
19	Poor	54	M	>2000	–	–/–	<2.6	8	+	18	47	ETV	49	F	–	Compatible	+	+	1000
20	Poor	63	M	1740	–	–/+	<2.6	12	–	17	42	LAM + ADV	36	M	+	Identical	–	–	0.2
21	Poor	58	M	35	–	–/+	<2.6	16	–	16	19	ETV	33	F	+	Identical	–	–	0.3
22	Poor	61	M	>2000	–	–/+	2.9	15	+	68	5	LAM	26	M	+	Identical	–	–	<0.1

NA nucleos(t)ide analogue, *LAM* lamivudine, *ADV* adefovir dipivoxyl, *ETV* entecavir, *HBcAb* anti-HBc antibody, *HBsAb* anti-HBs antibody

Table 5 Patient characteristics according to vaccine responsiveness in LC (univariate analysis)

N	Good responders 9	Poor responders 13	*p* value
Recipient related factors			
Age at OLT	50 (47–55)	54 (46–59)	0.546
Sex (male)	8 (88 %)	11 (84 %)	0.774
Time of vaccination (months after OLT)	42 (26–50)	30 (17–61)	0.442
HBsAg at OLT (≥1500 IU/l)	6 (66 %)	8 (61 %)	0.805
HBeAg positive at OLT	6 (66 %)	5 (38 %)	0.190
HBV DNA at OLT (≥3.7 logcopies/mL)	4 (44 %)	4 (30 %)	0.513
MELD at OLT	15 [12–18]	15 [8–19]	0.480
Child-Pugh score at OLT	10 [8–10]	9 [6–11]	0.845
HCC at OLT (+)	6 (66 %)	9 (69 %)	0.899
Anti-HBs antibody titer at the start of vaccination	18.6 (6.4–34.6)	17.4 (5.9–37.1)	0.920
Nucleos(t)ide analogue (LAM/LAM + ADV/ETV)	6/3/0	8/3/2	0.312
Tacrolimus/cyclosporinA	6/3	11/1#	0.148
Tacrolimus level (ng/mL)	4.7 (3.0–5.6)	3.8 (2.9–5.8)	0.744
Donor-related factors			
Age at OLT	48 (47–51)	33 (25–48)	0.019*
Sex (M)	2 (22 %)	7 (53 %)	0.138
ABO (identical)	3 (33 %)	9 (69 %)	0.093
Blood relation (no)	7 (77 %)	1 (7 %)	<0.001*
Anti-HBs antibody titer (>100)	6 (66 %)	3 (23 %)	0.038*
Anti-HBc antibody (+)	6 (66 %)	5 (38 %)	0.190
Anti-HBc(+)/anti-HBs(+)	6 (66 %)	4 (30 %)	0.093
Anti-HBc(+)/anti-HBs(−)	0 (0 %)	1 (7 %)	0.297
Anti-HBc(−)/anti-HBs(+)	0 (0 %)	0 (0 %)	–

MELD Model for End-stage Liver Disease, *HCC* hepatocellular carcinoma, *LAM* lamivudine, *ADV* adefovir dipivoxyl, *ETV* entecavir

One patient received no calcineurin inhibitor

Table 6 Multiple logistic analysis of factors associated with good responses to HBV vaccine in LC

N	Odds ratio	95 % CI	*p* value
Age at OLT (>47)	5.4	0.300–214.000	0.244
Blood relation (no)	29.4	2.551–984.110	0.005*
Anti-HBs antibody titer (>100)	5.0	0.343–149.947	0.233

Note: Variables significant at $p < 0.05$

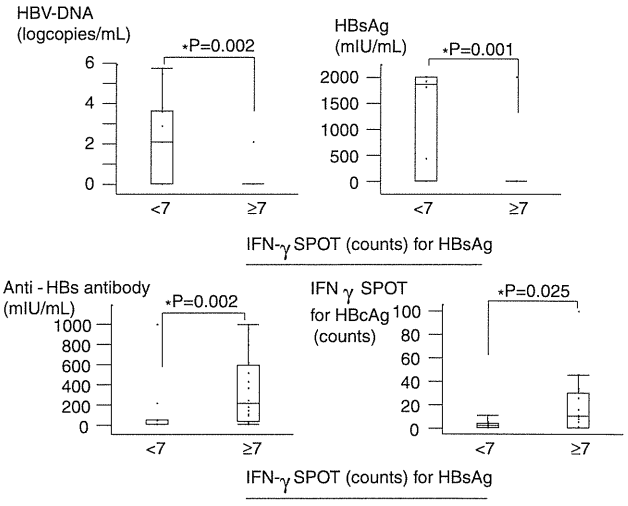

Fig. 2 The clinical characteristics of the non-OLT patients with strong HBsAg-specific T cell interferon-γ response. The clinical characteristics of the non-OLT patients showing strong HBsAg-specific T cell immune responses by enzyme-linked immunospot (ELISPOT) assay are shown. Those patients with stronger HBsAg-specific CD4 T cell IFN-γ response (equal or more than the median; 7 spots) showed lower HBV DNA, lower HBsAg, higher anti-HBs antibody titer, and higher HBcAg-specific immune responses

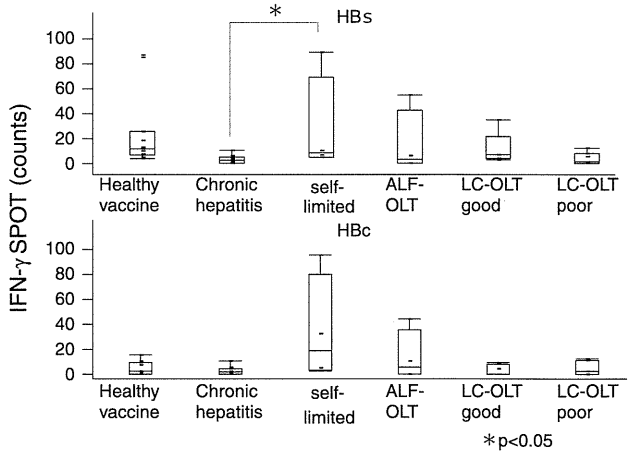

Fig. 3 Cellular immune responses against HBsAg including OLT patients. The number of spots due to interferon-γ response in the ELISPOT assay for HBsAg (*upper figure*) and HBcAg (*lower figure*) is shown. *1* Healthy vaccine: healthy controls who were positive for anti-HBs antibodies with HBV vaccine ($n = 11$). *2* Chronic hepatitis: chronic hepatitis B patients ($n = 10$). *3* Self-limited: self-limited acute hepatitis B patients who showed serum anti-HBs antibody-positive/HBcAb-positive with no HBsAg or HBV-DNA ($n = 5$). *4* ALF-OLT: post-OLT acute liver failure patients ($n = 4$). *5* LC-OLT good: post-OLT liver cirrhosis patients who showed good response to vaccine ($n = 8$). *6* LC-OLT poor: post-OLT liver cirrhosis patients who showed poor response to vaccine ($n = 7$). Values are plotted as median (range)

therapies. We previously have shown that long-term LAM with short-term, high-dose HBIg followed by low-dose HBIg (sufficient to maintain an anti-HBs antibody titer of >10 mIU/mL) is cost-effective and powerful enough to control HBV recurrence after LDLT [13]. With this cost-saving method, no clinical evidence of HBV recurrence has been seen.

In 2000, Sanchez-Fueyo et al. [21] reported an 82 % response to HBV vaccination after OLT. These researchers

used three cycles of double-dose recombinant HBsAg vaccine for immunization over 6 months, with a target antibody titer of >10 mIU/mL. The cohort included six acute infected patients and 11 chronic carriers. However, recent reports show that chronic HBV carrier recipients did not respond well, with response rates ranging from 7.7 to 12.5 % [22, 23]. Acute HBV-infected patients who underwent OLT were often positive for the anti-HBs antibody even before OLT, with strong immune responses. Such patients might be expected to respond well to vaccination, since these individuals (unlike chronic carriers) have not developed a tolerance to HBV. In our patients, five acute infected patients showed good responses to vaccination, responding after a median of only four vaccinations. These results indicate that while acute HBV-infected patients are good candidates for HBV vaccination post-OLT; chronic HBV carriers are poorer candidates for this protocol. However, as some HBV carriers did respond to vaccination; further studies should be performed to clarify the differences between the good and poor responders.

Several reports have identified the differences between good responders and poor responders in non-HBV-infected patients who received HBcAb-positive donor livers. Lacking previous HBV exposure, these recipients should not have developed tolerance to the virus and so should have been good responders. Of these, good responses were seen in pediatric cases where the recipients had higher anti-HBs antibody titers at the time of OLT and lower tacrolimus levels at the time of vaccination [24]. The present study revealed that repeated vaccine administration resulted in successful immunization in 40 % of the LC-OLT recipients. For these recipients, the strength of the response did not correlate with recipient characteristics, not even with age, one of the most important factors for successful immunization [25]. In contrast, the characteristics of the donor were important. The good responders' donors were relatively high in age, non-blood-related and had high anti-HBs antibody titers before donation. Note that, in our trial, the term "non-blood-related donor" indicates the spouse of the recipient, since deceased donor liver transplantation is not widely accepted in Japan [26]. The donors with high-titer anti-HBs antibody probably were infected with HBV by the recipients after their marriage, resulting in the anti-HBs antibody boost. These donors' immune systems should not have developed tolerance to the virus. This elevated immunity might be the reason why our patients had relatively better outcomes following vaccination than those of previous reports [27]. Adoptive immune transfer of HBV-specific immune response could be possible [28]. For successful transfer of immune memory to the recipients, the anti-HBs antibody titer of the donors should be high, and vaccine-induced anti-HBs antibody might be less effective than antibodies produced in a previous self-limited infection. Luo et al. [29] have shown that a particularly high anti-HBs antibody titer (>1000 IU/L) in the donor is essential for adoptive immune transfer. The results of the present study suggest that HBV vaccination of non-blood-related living donor candidates having a lower anti-HBs antibody titer (<100 mIU/mL) might facilitate improved vaccine response post-OLT in LC recipients.

The present study of HBV vaccine efficacy in ALF-OLT and LC-OLT patients revealed that the vaccine response depended on the immune tolerance to the virus in both recipients and donors. The liver is the biggest immune organ in the abdomen and so can play a critical role in immune responses. Multiple populations of non-hematopoietic liver cells, including sinusoidal endothelial cells, stellate cells located in the subendothelial space, and liver parenchymal cells, take on the roles of antigen-presenting cells [30]. The viral-specific immune competence of the grafted liver might overcome the general immunotolerance to the virus in chronic HBV carriers.

In conclusion, patients who received OLT due to acute infection of HBV were good candidates for HBV vaccination. The chronic HBV carrier recipients who received livers from donors who were non-blood-related (i.e, the recipient's spouse) and who harbored high anti-HBs antibody titers were the best candidates for HBV vaccine administration. Vaccine-induced, HBV-specific immune responses were strong enough to induce not only humoral but also cellular responses in vitro.

Acknowledgments We thank Taiko Kameyama, Asuka Maeda, Chizuru Mori, and Mayumi Honda for carrying out the ELISPOT assay experiments at our institute. Toshie Ishii assisted in the collection of the clinical data and assembly of the data files.

Conflict of interest The authors declare that they have no conflict of interest.

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Clinical & Experimental Immunology
The Journal of Translational Immunology

Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/cei.12247

Genetic variants of immunoglobulin γ and κ chains influence humoral immunity to the cancer-testis antigen XAGE-1b (GAGED2a) in patients with non-small cell lung cancer

J. P. Pandey,* A. M. Namboodiri,* Y. Ohue,§ M. Oka§ and E. Nakayama†
*Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC, USA, §Department of Respiratory Medicine, Kawasaki Medical School, and †Faculty of Health and Welfare, Kawasaki University of Medical Welfare, Kurashiki, Japan

Accepted for publication 30 November 2013
Correspondence: J. P. Pandey, Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC 29425-2230, USA.
E-mail: pandeyj@musc.edu

Summary

GM (γ marker) allotypes, genetic variants of immunoglobulin γ chains, have been reported to be associated strongly with susceptibility to lung cancer, but the mechanism(s) underlying this association is not known. One mechanism could involve their contribution to humoral immunity to lung tumour-associated antigens. In this study, we aimed to determine whether particular GM and KM (κ marker) allotypes were associated with antibody responsiveness to XAGE-1b, a highly immunogenic lung tumour-associated cancer-testis antigen. Sera from 89 patients with non-small cell lung cancer (NSCLC) were allotyped for eight GM and two KM determinants and characterized for antibodies to a synthetic XAGE-1b protein. The distribution of various GM phenotypes was significantly different between XAGE-1b antibody-positive and -negative patients ($P = 0{\cdot}023$), as well as in the subgroup of XAGE-1b antigen-positive advanced NSCLC ($P = 0{\cdot}007$). None of the patients with the GM 1,17 21 phenotype was positive for the XAGE-1b antibody. In patients with antigen-positive advanced disease, the prevalence of GM 1,2,17 21 was significantly higher in the antibody-positive group than in those who lacked the XAGE-1b antibody ($P = 0{\cdot}026$). This phenotype also interacted with a particular KM phenotype: subjects with GM 1,2,17 21 and KM 3,3 phenotypes were almost four times (odds ratio = 3·8) as likely to be positive for the XAGE-1b antibody as the subjects who lacked these phenotypes. This is the first report presenting evidence for the involvement of immunoglobulin allotypes in immunity to a cancer-testis antigen, which has important implications for XAGE-1b-based immunotherapeutic interventions in lung adenocarcinoma.

Keywords: cancer-testis antigen, GM/KM allotypes, humoral immunity, non-small cell lung cancer, XAGE-1b (GAGED2a)

Introduction

Genetic variants of immunoglobulin G (IgG) heavy chains are called GM allotypes. They are encoded by three very closely linked genes – immunoglobulin heavy chain G1 (*IGHG1*), *IGHG2* and *IGHG3* – on chromosome 14q32. They are expressed on the constant regions of γ1, γ2 and γ3 chains. There are striking qualitative and quantitative differences in the distribution of GM allotypes among different racial groups. In addition, there is almost complete linkage disequilibrium between particular GM determinants within a race, and every major racial group is characterized by a distinct array of GM haplotypes [1,2]. Using hypothesis-driven candidate gene approaches, several studies have identified particular GM genes/genotypes as risk factors for many malignant diseases [2–7]. In lung cancer, a highly significant association was found between the GM 1,2 13,15,16,21 phenotype and susceptibility to this malignancy in a Japanese population [8]. The mechanism(s) underlying this association is not known.

One mechanism underlying the reported GM gene–lung cancer association could involve the contribution of GM determinants to humoral immunity to lung tumour-associated antigens, as GM genes are known to influence immunity to several self and non-self antigens, including tumour-associated antigens mucin 1 and human epidermal

