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A multi-center survey of hospital charges for hemophilia and the related diseases in Japan

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Key words: hospital charges for hemophiliacs, Diagnosis Procedure Combination (DPC), comprehensive costing, inhibitor, total infusion dose

We estimated the hospital charges for the patients with hemophilia in eight institutions enlisted in this study, and discussed the characteristic features and points at issue regarding the comprehensive costing system using the Diagnosis Procedure Combination(DPC), when adopted. We calculated the total days of hospital admission and total charges based on the data supplied from the institutions during the period between April 2002 and March 2004. We analyzed various factors such as types of disease, patient's body weight, the presence or absence of inhibitors and whether or not the patients had been treated surgically.

The total days of hospital admission and the mean hospital charges per day(MHCD) varied among the institutions ranging from 124,110 yen to 220,080 yen, and the MHCD appeared to increase in accordance with the patient's body weight and the titer of inhibitors as well. Therefore, the comprehensive costing system using DPC would not be beneficial to the institutions, and its adoption appears to be difficult at this stage of investigation.

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Peptide-Loaded Dendritic-Cell Vaccination Followed by Treatment Interruption for Chronic HIV-1 Infection: A Phase 1 Trial

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Immune response enhanced by therapeutic HIV-1 vaccine may control viral proliferation after discontinuation of highly active antiretroviral therapy (HAART). Although which strategies for therapeutic vaccination are feasible remains controversial, application of dendritic cells (DCs) as a vaccine adjuvant represents a promising approach to improving deteriorated immune function in HIV-1-infected individuals. The safety and efficacy of DC-based vaccine loaded with HIV-1-derived cytotoxic T lymphocytes (CTL) peptides were thus investigated in this study. Autologous DCs loaded with seven CTL peptides with HLA-A*2402 restriction were immunized to four HIV-1-infected individuals under HAART. In terms of safety, peptide-loaded DCs were well tolerated, and only mild local and general symptoms were observed during vaccine administration. ELISPOT assays to detect IFN-γ production in $CD8^+$ lymphocytes revealed a limited breadth of responses to immunized peptides in two of four participants, but no response in the remaining two participants. Differences in immunological response might be attributable to the fact that responders displayed higher nadir CD4 counts before starting HAART and were immunized with a larger number of DCs per reactive peptide than non-responders. Discontinuation of HAART after vaccination failed to lower viral set points compared to those before starting HAART. This early outcome warrants further exploration to elucidate the therapeutic value of vaccination with DCs in HIV-1 infection. ***J. Med. Virol. 78: 711–718, 2006.*** © 2006 Wiley-Liss, Inc.

KEY WORDS: HIV-1; vaccine; HAART; treatment interruption

INTRODUCTION

Although highly active antiretroviral therapy (HAART) has significantly improved prognosis for HIV-1 infection, life-long therapy remains a requirement for continuous viral suppression [Ramratnam et al., 2000; Siliciano et al., 2003]. Long-term toxicity of HAART is therefore of medical concern and the economic cost of HAART has become a major social problem. These issues have facilitated attempts at strategic or structured treatment interruption (STI). However, successful results have not been obtained in patients starting HAART in the chronic phases, as HIV-1-specific immunity is already exhausted at the moment of treatment interruption [Oxenius et al., 2002; Fagard et al., 2003; Kaufmann et al., 2004].

Several lines of evidence have revealed that cytotoxic T lymphocytes (CTLs) play a critical role in control of HIV-1 proliferation, and that maintenance of CTL function during chronic infection requires the presence of $CD4^+$ helper T cells [Borrow et al., 1994; Koup et al., 1994; McMichael and Rowland-Jones, 2001]. However, HIV-1 selectively infects and destroys HIV-1-specific $CD4^+$ T cells, and causes quantitative and qualitative

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TABLE I. Baseline Subject Characteristics

	Subject			
	1	2	3	4
Age (years)	39	46	52	40
Sex	M	M	M	M
Duration of known seropositivity (months)	66	39	46	67
Duration of HAART (months)	65	37	42	64
HAART menu[a]	AZT + 3TC + EFV	AZT + 3TC + EFV	AZT + 3TC + EFV	d4T + 3TC + NFV
CD4 counts (/μl)				
Before HAART	164	216	50	2
At enrollment	453	658	330	340
Viral load (copies/ml)				
At enrollment	<50	<50	<50	<50
Preparation of vaccine				
Number of PBMCs obtained by leukopheresis	7.6×10^9	9.4×10^9	6.8×10^9	9.7×10^9
Number of DCs used for each vaccination	$0.7–1.2 \times 10^7$	$0.9–1.4 \times 10^7$	$1.0–1.5 \times 10^7$	$1.2–1.8 \times 10^7$
Amino acid sequences of epitope portions[b]				
Gag28	3R	3R, 7L[c]	3R	3R, 7V[c]
Gag296	wt	wt	wt	wt
Nef138	2F	5C[c]	2F	2F
Env584	4G[c]	4Q	4K, 7R, 11L[c]	wt and 4K[c]

[a]AZT, azidothymidine; 3TC, lamivudine; EFV, efavirenz; NFV, nelfinavir.
[b]wt (wild-type) represents amino acid sequences are identical to those of SF-2. Others represent amino acid positions of substitution and the substituted amino acids (see Table II).
[c]These substitutions were not included in peptides used in this study.

impairment of HIV-1-specific immunity, as shown by us and other groups [Watanabe et al., 2001; Kawamura et al., 2003]. When HAART is started and viral proliferation is controlled, destruction of $CD4^+$ T cells stops and naive lymphocytes are provided from the thymus. The immune system, however, is unable to produce and maintain HIV-1-specific immunity due to a loss of antigen stimuli under HAART. Treatment interruption in patients who start HAART in the chronic phase thus results in unfavorable outcomes.

Given this pathogenesis of HIV-1 infection, a therapeutic HIV-1 vaccine that is administered during HAART and potentiates HIV-1-specific immunity would theoretically offer a feasible strategy for achieving better viral control after STI. To test this hypothesis, we conducted a phase 1 clinical trial in which autologous dendritic cells (DCs) loaded with HIV-1-derived CTL epitope peptides were administered to four HIV-1-infected individuals and HAART was discontinued thereafter. DCs were used as highly specialized antigen-presenting cells that not only restore qualitative impairment of CTLs, but also stimulate naive $CD8^+$ T cells newly provided from the thymus during HAART [Banchereau et al., 2000]. We report herein the safety and efficacy of this DC-based therapeutic vaccine in addition to clinical outcomes after interruption of HAART.

MATERIALS AND METHODS

Participants

Subjects comprised four men with chronic HIV-1 infection and HLA genotype A*2402. All participants were under HAART with undetectable viral loads (VL; <50 copies/ml) for ≥1 year before enrollment. The institutional ethics committee approved this clinical trial and all participants provided written informed consent. Baseline characteristics are summarized in Table I.

Synthetic Peptides

Clinical-grade synthetic peptides (Table II) used for vaccination and ELISPOT assay were purchased from Multiple Peptide Systems (San Diego, CA). Gag(1–115) comprises a pool of 12- to 17-mer peptides with 10 amino acid overlaps that cover the whole Gag protein (subtype B consensus sequence) but do not include peptides containing Gag28 and Gag296 epitopes. Gag overlapping peptides were purchased from Operon Biotechnologies (Huntsville, AL). CMV-pp65 [Kuzushima et al., 2001] and EBV-TL9 [Lee et al., 1997] are both HLA-A24-restricted epitopes derived from Cytomegalovirus and Epstein-Barr virus and were purchased from Sigma-Genosys Japan (Ishikari, Japan).

TABLE II. A*2402-restricted CTL Epitope Peptides Used in This Study

			Peptides used in this study	
Protein	Epitope	Amino acid position	Designation	Sequence
Gag	Gag28	28–36	Gag28-wt	KYKLKHIVW
			Gag28-3R	KYRLKHIVW
	Gag296	296–306	Gag296	RDYVDRFYKTL
Nef	Nef138	138–147	Nef138-wt	RYPLTFGWCF
			Nef138-2F	RFPLTFGWCF
Env	Env584	584–594	Env584-wt	RYLRDQQLLGI
			Env584-4Q	RYLQDQQLLGI

RNA Extraction, PCR Amplification, and Sequencing

Viral RNA was extracted from plasma and subjected to first and second polymerase chain reaction (PCR), as described previously [Furutsuki et al., 2004]. PCR primers for Nef and Env epitope portions have been described previously [Furutsuki et al., 2004], and other primers are listed below (all nucleotide positions are in accordance with the HIV-1 SF2 strain).

For Gag28 epitope, 1st PCR primer set: forward: 5′-CGCAGGACTCGGCTTGCTGAAG-3′ (691-712) reverse: 5′-GCTATGTCACTTCCCCTTGGTTC-3′ (1506-1484). For Gag28 epitope, 2nd PCR primer set: forward: 5′-GAGAGAGATGGGTGCGAGAGC-3′ (784-804) reverse: 5′-TCTCTAAAGCTTCCTTGGTGTC-3′ (1097-1076). For Gag296 epitope, 1st PCR primer set: forward: 5′-AAGTAATACCCATGTTTTCAG-3′ (1296-1316) reverse: 5′-CTAAAATTGCCTCTCTGCATC-3′ (1947-1927). For Gag296 epitope, 2nd PCR primer set: forward: 5′-CCAGATGAGAGAACCAAGG-3′ (1474-1492) reverse: 5′-ATCTGGGTTTGCATTTTGG-3′ (1783-1765). For reverse transcriptase region, 1st primer set: forward: 5′-ATGATAGGGGGAATTGGAGGTTT-3′ (2393-2415) reverse: 5′-TACTTCTGTTAGTGCTTTGGTTCC-3′ (3422-3399). For reverse transcriptase region, 2nd primer set #1: forward: 5′-GACCTACACCTGTCAACATAATTGG-3′ (2492-2516) reverse: 5′-TAATCCCTGCATAAATCTGACTTGC-3′ (3379-3355). For reverse transcriptase region, 2nd primer set #2: forward: 5′-GTACTTTAAATTTCCCCATTAGTCC-3′ (2543-2567) reverse: 5′-CAGTCCAGCTGTCTTTTTCTGGC-3′ (3316-3294). For protease region, 1st primer set: forward: 5′-AGACAGGYTAATTTTTTAGGGA-3′ (2074-2095) reverse: 5′-TATGGATTTTCAGGCCCAATTTTTGA-3′ (2716-2691). For protease region, 2nd primer set: forward: 5′-AGAGCCAACAGCCCCACCAG-3′ (2155-2174) reverse: 5′-ACTTTTGGGCCATCCATTCC-3′ (2618-2599).

Purified PCR products were either directly sequenced or subcloned into pGEM-T vectors (Promega, Madison, WI) and sequenced using an ABI Prism dye terminator cycle sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA) on a Perkin-Elmer ABI-377 sequencer.

Preparation of DCs

Leukocytes fractions were collected from participants by leukopheresis of blood samples, and peripheral blood mononuclear cells (PBMCs) were purified through a ficoll-hypaque gradient. Obtained PBMCs were aliquoted into cryotubes, and stored at −150°C until use. For induction of immature DCs, frozen PBMCs were thawed, suspended in PBS and incubated in plastic dishes for 30 min at 37°C. Adherent cells were then cultured in RPMI medium (HyClone, Logan, UT) containing 10% human AB serum (COSMO BIO, Tokyo, Japan), 50 ng/ml GM-CSF (PeproTech, Rocky Hill, NJ), and 50 ng/ml of recombinant human interleukin 4 (PeproTech). After 6–7 days of culture, TNF-α (PeproTech) (50 ng/ml) and 7 CTL epitope peptides (10 μM each) were added and peptide-loaded mature DCs were harvested the next day. Cells were resuspended in 1 ml of saline and kept on ice until inoculation into participants. All procedures were conducted in a dedicated facility based on GCP as defined by the Japanese Ministry of Health, Labor, and Welfare.

Vaccination and Interruption of HAART

DCs loaded with HIV-1-specific epitope peptides were injected subcutaneously into axillary areas six times every 2 weeks. After the 6th vaccination, HAART was discontinued and clinical, immunological, and virological consequences were observed every week. If the HAART regimen contained efavirenz, nevirapine, or lamivudine, these antiretroviral agents were changed at least 2 weeks before treatment interruption to other agents with shorter half-lives. HAART was restarted when participants met any of the following criteria: VL > 50,000 copies/ml; VL > 5,000 copies/ml on three consecutive measurements; or CD4 counts <200/μl on two consecutive measurements.

ELISPOT Assay

For ELISPOT assay, PBMCs were aliquoted to 96-well multiscreen plates precoated with 5 μg/ml anti-IFN-γ monoclonal antibody (mAb) 1-D1K (Mabtech, Nacka Stand, Sweden). Peptides were added at concentrations of 10^{-6} M and incubated for 18 hr at 37°C. After washing wells, 100 μl of 1 μg/ml biotinylated anti-IFN-γ mAb 7-B6-1 (Mabtech) was added and incubated at room temperature for 90 min. After unbound mAb was removed, 100 μl of 1:1,000 diluted streptavidin-alkaline phosphatase conjugate (Mabtech) was added and incubated at room temperature for 60 min. Spots were developed using an alkaline phophatase conjugate substrate kit (BIO-RAD, Hercules, CA), and counted using a KS Elispot compact (Carl Zeiss, Oberkochen, Germany). Assays were conducted in triplicate and results were represented as mean numbers of spots per 10^6 PBMCs. When the number of spots was more than three-times the number in controls (PBMCs cultured without peptides), the response was considered significant.

RESULTS

Selection of CTL Epitope Peptides Derived From HIV-1

As immunogens for vaccination, we used HIV-1-derived peptides that were known to elicit strong CTL response and were restricted to HLA-A*2402 expressed in approximately 70% of the Japanese population (allele frequency of A*2402; 36.5%) [Tanaka et al., 1996]. The selected CTL epitope portions with A*2402 restriction were amino acid positions from 28 to 36 of Gag(Gag28), from 296 to 306 of Gag(Gag296), from 138 to 147 of Nef (Nef138), and from 584 to 594 of Env (Env584) (Table II). Whereas amino acid sequences in the Gag296 epitope portion have been shown to be conserved, other

three-epitope portions have been reported to display amino acid mutations [Ikeda-Moore et al., 1997, 1998; Dorrell et al., 1999; Furutsuki et al., 2004]. Thus, for the three-epitope portions, both wild-type peptides (Gag28-wt, Nef138-wt, and Env584-wt) and one of the representative mutant peptides (Gag28-3R, Nef138-2F, and Env584-4Q) were selected (Table II). Sequence analysis of HIV-1 derived from the four enrolled participants revealed that at least two of four epitope portions displayed amino acid sequences identical to immunized peptides (Table I).

DC-Based Vaccine Administration and Treatment Interruption

The four men enrolled in this study displayed undetectable VL under HAART (Table I). Leukopheresis was used to collect $6.8-9.7 \times 10^9$ PBMCs from each participant, and $0.7-1.8 \times 10^7$ mature DCs were harvested for each vaccination without contamination by pathogens or reactivation of autologous HIV-1. Peptides were either loaded to DCs by mixture (Subjects 1 and 2) or separately (Subjects 3 and 4), and peptide-loaded DCs were injected subcutaneously to areas near the axilla in two to three divided doses. During the course of six vaccinations in the four participants, subcutaneous bleeding (n = 1), erhythema at the injection site (n = 1), and general malaise (n = 1) were reported as local and generalized adverse events, all of which were non-serious and resolved without specific treatment (Table III).

Serum VLs were examined every week after treatment interruption and became positive above the detection limit of 50 copies/ml in all four participants, in weeks 3, 3, 1, and 2, respectively (Table III; Fig. 1). Subject 4 experienced fever at 38°C, myalgia, skin rash, and cervical lymph node swelling at 1 week after interruption, accompanied by mild liver dysfunction and thrombocytopenia, mimicking acute retroviral syndrome, and subsiding spontaneously within 2 weeks. All participants met criteria to restart HAART (at weeks 8, 4, 5, and 3, respectively) and VLs had been suppressed to undetectable levels by 11–30 weeks after restart of original HAART regimens. Differences between peak VL after treatment interruption and VL before start of HAART did not exceed 0.5 in $\log_{10}$ scale in all four participants (Table III). CD4 counts decreased after discontinuation of HAART in all participants to the level of approximately 200/μl (Fig. 1). After restarting HAART, CD4 counts in Subjects 2 and 4 gradually recovered, but those in Subjects 1 and 3 fluctuated at lower levels than prior to treatment interruption despite successful viral control by restarted HAART.

Immunological Analysis of Vaccines

HIV-1-specific CTL response to immunized peptides was evaluated by ELISPOT assay to detect IFN-γ-producing cells. Unseparated PBMCs were used for the assay, as preliminary experiments showed that IFN-γ production responding to both immunized peptides and control peptides was only seen in the $CD8^+$ population (data not shown). Significant responses to Nef138-wt were observed in Subjects 1 and 2, with weak responses to Nef138-2F in Subject 1 after the 5th vaccination (Fig. 2; black bars). Response in Subject 2 was

TABLE III. Clinical Outcomes of Vaccine Administration and Interruption of Antiretroviral Therapy

	Subject			
	1	2	3	4
CD4 counts (/μl)				
At 1st vaccination	512	310	520	428
Nadir after treatment interruption	257	252	181	213
Decrease in CD4 counts[a]	−255	−58	−339	−215
Viral load ($\log_{10}$ [copies/ml])				
Before start of HAART	4.08	5.15	4.15	5.23
Peak after treatment interruption	4.00	5.04	4.58	5.26
Reduction of VLs[b]	0.08	0.11	−0.43	−0.03
First detectable VLs after interruption (weeks)	3	3	1	2
Duration of interruption (weeks)	10	18	6	5
Adverse events				
During vaccination	Subcutaneous bleeding at injection site		General malaise	Erythema at injection site
After interruption of HAART				Fever, lymph node swelling, thrombocytopenia, elevated liver enzyme
HAART during 2 weeks before interruption	AZT + ddC + NFV	AZT + ddC + NFV	AZT + ABC + NFV	d4T + ABC + NFV
Drug-resistant mutations				
Reverse transcriptase region	None	None	None	None
Protease region	None	None	M36J (4w) none (6w)	None

[a]CD4 count at nadir after treatment interruption subtracted from count at 1st vaccination.
[b]VL before start of HAART subtracted from VL at peak after treatment interruption.

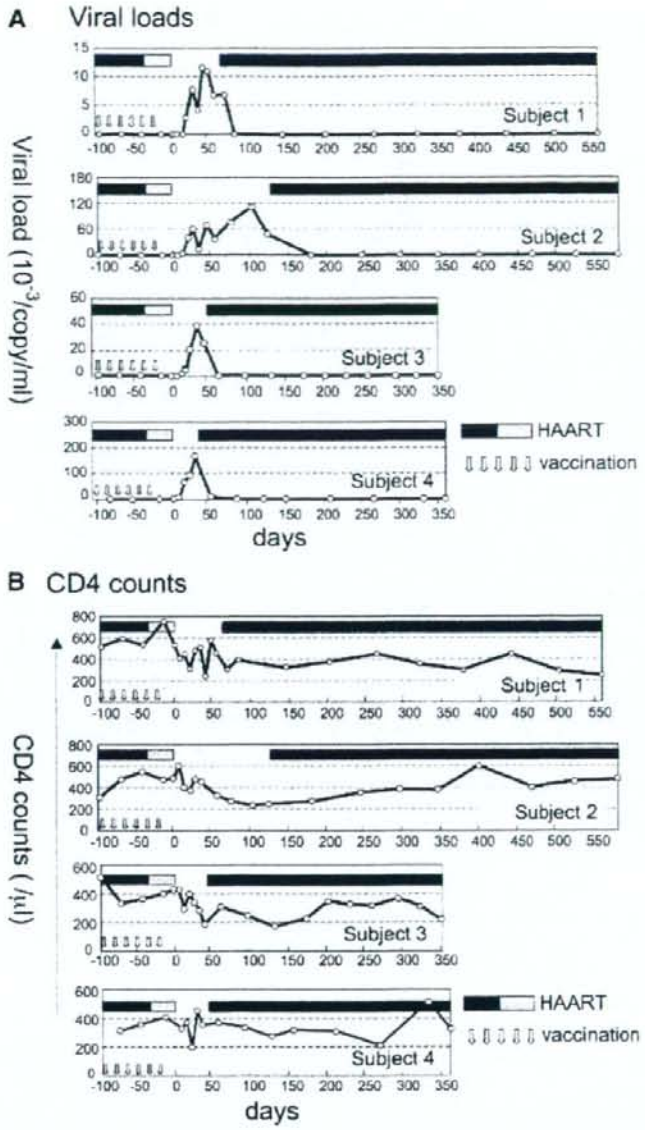

Fig. 1. Clinical courses of the four subjects. Viral loads (**A**) and CD4 counts (**B**) of the four subjects during vaccination (downward arrows) are described together with duration of treatment interruption and restart of HAART. Black bars represent duration under original HAART regimens and gray bars represent duration under alternative HAART regimens to avoid drug resistance.

specifically induced by DC-based vaccine, as no responses to control peptides of Gag(1–115), CMV-pp65, or EBV-TL9 were detected. In Subject 1, however, response was also observed to control peptides of Gag(1–115) and EBV-TL9 after the 5th vaccination, suggesting that this response to Nef138-wt and Nef138-2F included non-specific stimuli by DC injection. When HAART was discontinued and autologous virus rebounded, specific responses in Subjects 1 and 2 were induced to Nef138-wt and Nef138-2F in addition to Gag(1–115), whereas

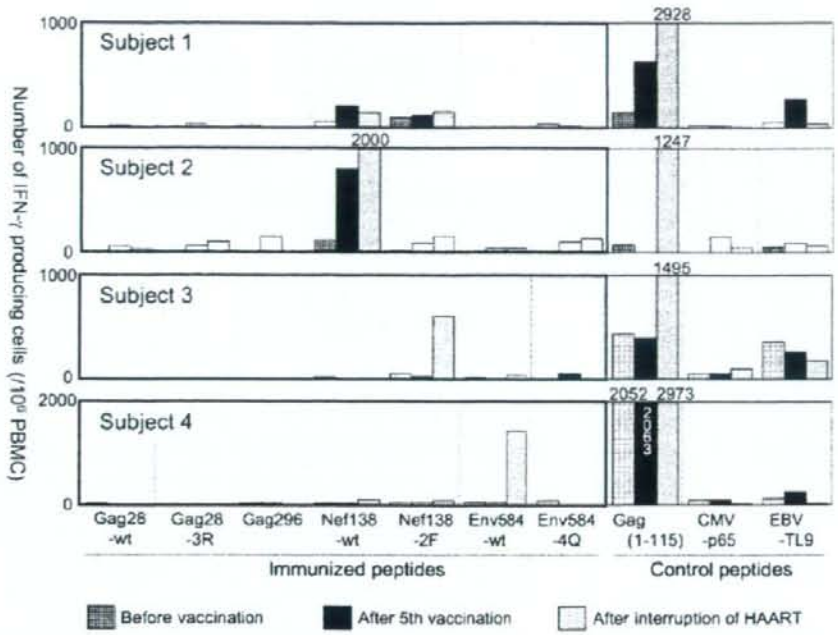

Fig. 2. Peptide-specific responses in PBMCs of vaccines. PBMCs collected before vaccination (shaded bars), after 5th vaccination (black bars) and 4 weeks after treatment interruption (gray bars), were incubated with immunized peptides: Gag28-wt, Gag28-3R, Gag296, Nef138-wt, Nef138-2F, Env584-wt, and Env584-4Q in addition to control peptides: Gag(1–115), CMV-pp65, and EBV-TL9. Response to each peptide was analyzed using ELISPOT assay detecting IFN-γ-producing cells. Specific response was calculated by subtracting number of IFN-γ-producing cells without peptides from number of cells with each peptide, and the subtracted number of cells was represented as per 10^5 PBMCs. When absolute numbers of IFN-γ-producing cells with peptides were less than three times numbers of cells without peptides, the response was considered as background and represented with white bars. To show details of data, some bars are scaled out and the specific response is represented by actual numbers of spots per 10^5 PBMCs.

no significant responses were observed for other immunized peptides.

The limited breadth of response in Subjects 1 and 2 to immunized peptides raised the possibility that differences in avidity between immunized peptides and HLA-A*2402 molecules affected the results because seven peptides were loaded by mixture into DCs for these participants. Avidity of the seven peptides was thus tested using a T2-A24 stabilization assay [Foung et al., 1986; Kuzushima et al., 2001], revealing that Env584-wt, Env584-4Q, Nef138-wt, and Nef138-2F bind HLA-A*2402 with relatively high avidity, whereas Gag28-3R binds with moderate avidity, and both Gag296 and Gag28-wt bind with low avidity (data not shown). Based on this result, each peptide for Subjects 3 and 4 was incubated with DCs separately ($\sim 1.4 \times 10^6$ DCs/peptide) and used for vaccination. However, injection of separately-loaded DCs did not induce specific response to any of the seven immunized peptides, despite the fact that rebound of autologous HIV-1 after treatment interruption induced strong responses to Nef138-2F, Env584, and Gag(1–115). We also conducted tetramer-binding assay using Nef138-wt-tetramer and ELISPOT assay using autologous DCs as antigen-presenting cells to amplify IFN-γ production, but could not find peptide-specific population or response in Subjects 3 and 4 (data not shown).

Drug-Resistance Mutations

Since one of the concerns regarding interruption of HAART is the potential emergence of drug-resistance mutations, we sequenced reverse transcriptase and protease genes of HIV-1 derived from plasma before start of HAART and 4 weeks after treatment interruption, when VLs were detectable in all participants (7,100, 58,000, 24,000, and 100,000 copies/ml, respectively). Subject 3 displayed a nucleotide substitution at position 108 in the protease regions on population sequencing, which would result in an amino acid change from methionine to isoleucine at position 36 (Table III). Sequences of clones obtained from the PCR product revealed that five of five clones displayed the M36I mutation. HIV protease genes in the plasma of this participant were further sequenced at 6 weeks after treatment interruption (29,000 copies/ml), but no M36I mutation were identified in any of the eight clones sequenced. No nucleotide substitutions were found in protease genes from the other three participants, or in reverse transcriptase genes from all participants.

DISCUSSION

After DC-based vaccination of the four subjects, immune responses to Nef138-wt in Subjects 1 and 2, and to Nef138-2F in Subject 1 were observed, whereas no detectable responses were obtained in other peptides. The results from Subjects 1 and 2 demonstrating limited breadth of response led us to consider the possibility that differences in avidity between HLA-A*2402 molecules and each peptide caused preferential presentation of Nef138 epitopes, as seven peptides were added to DCs in mixture. In fact, T2-A24 stabilization assay revealed that Gag epitopes displayed lower avidity to HLA-A*2402 than Nef138 and Env584 peptides. Thus, in Subjects 3 and 4, the seven peptides were incubated with DCs in separate wells and mixed together before vaccination, but no significant responses were observed to any of these peptides. One explanation for this observation is that when approximately 1×10^7 DCs were divided among seven peptides (approximately 1.4×10^6 cells/peptide), the numbers of DCs was too small to provide sufficient stimuli to CTLs in vivo. Although Yu et al. [2004] reported that 1.0×10^6 autologous DCs loaded with glioma-derived peptides could elicit systemic cytotoxicity in cancer patients, the number of DCs in the present cases might have been insufficient to elicit specific response from HIV-1-infected individuals. Another explanation is that Subjects 3 and 4 displayed lower nadir CD4 counts before starting HAART (50/μl and 2/μl, respectively) than Subjects 1 and 2 (164/μl and 216/μl, respectively). In untreated HIV-1-infected individuals, $CD4^+$ T cells are continuously destroyed during all stages of HIV-1 infection, causing not only quantitative, but also qualitative abnormalities in HIV-1-specific immunity. These abnormalities are carried over even after CD4 counts are normalized by the initiation of HAART. In fact, Lange et al. [2003] reported that responses to immunization of tetanus and diphtheria toxoids in chronically HIV-1-infected patients under HAART correlate with previous nadir CD4 counts, but not with current circulating CD4 counts. This kind of impaired immune function in HIV-1-infected individuals under HAART may also explain the limited breadth of immune response in Subjects 3 and 4.

In terms of safety, peptide-loaded DCs were well tolerated, and only mild local and general symptoms were observed during vaccine administration, with only one episode of acute retroviral syndrome after STI. Since treatment interruption sometimes causes viral mutation resulting in antiretroviral drug resistance [Schweighardt et al., 2002; Metzner et al., 2003; Tremblay et al., 2003], all participants changed from antiretroviral agents that are known to be susceptible to resistance mutations to other agents with short-half lives before STI. However, an M36I mutation in a protease region was transiently detected in Subject 3 when VL rebounded 4 weeks after treatment interruption, and disappeared 6 weeks after interruption. M36I mutation is regarded as one of the minor resistance mutations that can appear after emergence of major resistance mutations. We cannot determine the mechanism underlying this transient appearance of M36I in Subject 3, but replication of mono- or oligoclonal HIV-1 from reservoir cells may be responsible.

In this study with a small number of participants, DC-based vaccine elicited a limited breadth and strength of immune response, and treatment interruption failed to control rebound of viral proliferation. Several groups have tried similar therapeutic vaccines to interrupt antiretroviral therapy in both humans [Hejdeman et al., 2003; Bostrom et al., 2004; Harrer et al., 2005; Kinloch-de Loes et al., 2005; Tubiana et al., 2005; Wu et al., 2005] and macaques [Lisziewicz et al., 2005] using recombinant proteins or genes expressing HIV-1 proteins, and have reported various results of specific immune reaction and clinical outcomes after treatment interruption. Although the question as to which strategy for therapeutic vaccination is suitable for successful treatment interruption remains controversial, application of DCs as vaccine adjuvant appears theoretically attractive to improve deteriorated immune function in HIV-1-infected individuals. In agreement with this concept, therapeutic vaccine using DCs in cancer treatment has been shown to result in better tumor regression compared to vaccines using peptide alone, viral vectors or tumor cells [Banchereau and Palucka, 2005]. Recently, two groups reported preliminary results of DC-based therapeutic vaccine in HIV-1-infected patients using autologous HIV-1 as immunogens in untreated [Lu et al., 2004] and treated patients [Garcia et al., 2005]. Garcia et al. showed that DC-based HIV-1 vaccine in patients under HAART did not elicit specific immune responses, although the vaccine suppressed viral rebound in 4 of 12 vaccines after treatment interruption. Our result thus provides encouraging evidence that DC-based vaccines can induce specific immune response, albeit insufficient to suppress viral rebound, in patients under HAART. These early outcomes warrant further exploration to establish the therapeutic value of vaccination with DCs in HIV-1 infection.

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◆原　著◆

インヒビター保有血友病患者における遺伝子組換え活性型血液凝固第VII因子製剤（注射用ノボセブン®）の長期的安全性および有効性：5年間の市販後調査中間解析報告

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遺伝子組換え活性型血液凝固第 VII 因子製剤（rFVIIa）の使用成績調査について，5 年間の長期有効性および安全性に関する中間解析結果をまとめた．先天性および後天性血友病患者 102 例 1,580 出血エピソードが報告された．全出血エピソードの中で 12 時間以内に止血効果が認められた，著効あるいは有効と評価された出血エピソードの割合（有効率）は 69.6%であった．また，8 時間以内に止血効果が認められた著効の割合は，既報の臨床成績（31.2%）のほぼ 2 倍（60.9%）であった．全出血エピソードのうち，3 つの要件（初回投与量 90 μg/kg 以上，出血から初回投与までの時間 3 時間以内，平均投与間隔 3 時間以内）を全て満たした群（推奨治療条件群）の有効率は 82.4% で，その他の群（非推奨治療条件群）の有効率 44.4%に比し有意に高かった．しかし，推奨治療条件を満たした治療エピソードは全体の約 40% に過ぎず，rFVIIa の有効性をさらに高めるためには 3 つの要件を満たすことの重要性が示唆された．安全性について，副作用は 20 例 42 件が報告された．重篤な副作用は 3 例 4 件報告されたが，いずれも本剤との明らかな関連性は認められなかった．

Key words: rFVIIa, haemophilia, inhibitor, bleeding episode

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緒 言

先天性血友病は血液凝固第VIII因子または第IX因子の欠乏あるいは質的異常により内因系血液凝固機序が障害される遺伝性出血性疾患である．止血管理には第VIII因子製剤または第IX因子製剤による補充療法を行うが，しばしば当該凝固因子に対する同種抗体（インヒビター）を産生する[1]．インヒビターの発現後はそれまでの補充療法の効果が失われるため，出血時には特殊な止血療法が必要となる．止血療法としては，大量の第VIII因子製剤または第IX因子製剤を投与して血漿中のインヒビターを中和し，第VIII因子活性または第IX因子活性を上昇させる方法，あるいは活性型プロトロンビン複合体製剤または活性型第VII因子製剤により，内因系凝固カスケードをバイパスして止血をはかる方法がある．

一方，凝固系に異常がない場合にも稀に各種の血液凝固因子に対する自己抗体の発現することがある．これらの自己抗体の中で最も高頻度に認められるのは，第VIII因子に対するインヒビターである[2]．これらの症例は，先天性血友病A患者と類似の臨床所見を示し，後天性血友病Aとも称される．後天性血友病Aの死亡率は15[2]～22%[3]と生命予後が悪い．また，先天性血友病では関節内出血が主な出血症状であるのに対し，後天性血友病では広範な皮下出血，筋肉内出血が多いことが特徴である．

遺伝子組換え活性型血液凝固第VII因子製剤（rFVIIa：注射用ノボセブン®，ノボ ノルディスク ファーマ㈱）は，第VIII因子または第IX因子に対するインヒビターを保有する血友病AまたはB患者の出血抑制を適応として日本では2000年3月に承認された．この適応症の中に後天性血友病が含まれるか否かが明確でなかったことから[4]，日本血栓止血学会は記載整備の要望書を厚生労働省に提出した．その結果，2004年11月には後天性血友病も適応症として明確に記載された．

rFVIIaは血管壁の損傷部位において組織因子と複合体を形成し，第X因子を活性化する．この活性型第X因子は引き続き，血管壁損傷部位に集積した活性化血小板膜上で活性型第V因子およびプロトロンビンとプロトロンビナーゼ複合体を形成し，トロンビンが生成される．また，rFVIIaは活性化血小板の表面上で組織因子非依存的に第X因子を直接活性化する機序も知られている[5]．これらの作用で生成されたトロンビンにより局所的にフィブリン形成が進み，血管壁の損傷部位に特異性の高い止血効果がもたらされると考えられている．

rFVIIaは承認時に10年間の再審査期間が指定され，現在，使用成績調査（以下，本調査）が実施されている．本稿では，2000年3月から2004年6月末までに収集したデータを基にrFVIIaの有効性，安全性および使用状況を中間解析した結果を報告する．

対象および方法

1. 対象と評価項目

ノボ ノルディスク ファーマ㈱と本調査の契約を締結した施設において，rFVIIa（注射用ノボセブン®1.2mgおよび4.8mg，ノボ ノルディスク ファーマ㈱）を投与された症例のうち，2000年3月から2004年6月末までに調査票が収集された症例を有効性評価の対象とした．また，安全性の検討では，本調査に加えて医療関係者から自発的に報告された副作用情報も評価対象とした．

出血エピソードごとに，出血日時，出血部位，投与日時，投与量，初回投与がなされた場所，投与時の症状および止血効果を記録した．日常診療の中で測定された一般臨床検査および凝固系の臨床検査項目として，APTT，PT，TAT，D-ダイマー，FDPおよびフィブリノゲンのデータを検討した．また，臨床検査値異常を含む有害事象，発現日，重篤度，治療，因果関係および転帰を検討した．

2. rFVIIaの有効性の評価

調査担当医師が，各出血エピソードにおける止血効果を，止血効果評価基準に従って「著効」，「有効」，「やや有効」および「無効」の4段階で評価した．この基準は，日本におけるrFVIIaの承認の根拠となった臨床試験（著者ら[6]）で使用された評価基準を採用した．

「著効」と「有効」に分類された両出血エピソード数が解析に含めた全出血エピソード数に占める割合（%）を有効率とした．なお，「著効」および「有効」と判断されたものは，rFVIIa投与からそれぞれ8時間以内および8～12時間に止血効果が認められた出血エピソードである．全体的な有効率のほか，全出血エピソードをrFVIIaの止血効果の判定に影響を与えると考えられる薬剤が併用された「rFVIIaと他剤併用治療群」と「rFVIIa単独治療群」に分け，それぞれの群についての有効率も算出した．rFVIIaの止血効果の判定に影響を与えると考えられる薬剤として，他の血液凝固因子製剤，非ステロイド系消炎鎮痛剤，ステロイド剤およびトラネキサム酸を含めた．

rFVIIa投与に関する各種パラメータ（出血部位，重症度，初回投与がなされた場所，初回投与量，出血から初回投与までの時間，平均投与間隔，投与回数）についての連続変数をカテゴリー化し，有効率を算出した．著者ら[6]，Parameswaranら[7]およびKeyら[8]の報告で本剤の止血効果に対して，初回投与量，出血から初回投与までの時間および平均投与間隔が重要と述べられていることから，「初回投与量が90 μg/kg以上」，「出血から初回投与までの時間が3時間以内」かつ「平均投与間隔が3時間以内」の3つの要件を全て満たす治療群（以下，推奨治療条件群）とそれ以外の治療群（以下，非推奨治療条件群）に分け，2群間で有効率を比較した．なお，本解析対象は，上記の全パラメータが得られた「rFVIIa単独治療群」および「rFVIIaと他剤併用治療群」の関節内，筋肉内および皮下出血とした．なお，本解析においては止血効果に最も大きな影響を与えると考えられる血液凝固因子製剤は他剤併用治療薬から除外した．

3. 統計解析

各パラメータごとにカテゴリー間の有効率の差をχ^2検定で比較した．「推奨治療条件群」と「非推奨治療条件群」の有効率に及ぼす影響についてロジスティック回帰分析を行った．また，有効性に影響を及ぼすと考えられる因子（初回投与量，出血から初回投与までの時間，平均投与間隔）についてもロジスティック回帰分析を行い検討した．有意水準は両側$p=0.05$とした．

結　果

1. 解析対象症例数および出血エピソード数

rFVIIaを投与された102例の1,580出血エピソードを解析対象とした．解析対象症例の内訳はインヒビターを保有する先天性血友病75例（血友病A 59例，血友病B 16例），インヒビターを保有しない先天性血友病3例（血友病A 2例，血友病B 1例），先天性第VII因子欠乏症1例，後天性血友病A 23例であった．

安全性は102例全例を対象として解析したが有効性については，i）適応外使用，ii）出血部位，投与日時，投与時の症状および効果判定が不明なもの，iii）定期補充療法，予備的補充療法，静脈内持続投与および試験投与（非出血時における薬物動態検査のための投与）を除いた，88例1,105出血エピソードを対象として解析した．内訳は，インヒビター保有の先天性血友病が71例1,060出血エピソード，後天性血友病Aが17例45出血エピソードであった．

全出血エピソードのうち半数弱の528出血エピソード（47.8%）が「rFVIIa単独治療群」で残りの577出血エピソード（52.2%）が「rFVIIaと他剤併用治療群」であった．

1,105出血エピソードのうち216出血エピソード（19.5%），また「rFVIIa単独治療群」528出血エピソードのうち104出血エピソード（19.7%）は本剤投与前の出血症状（スコア）が

重症であった．

2．有効性

有効性解析に含めた全出血エピソードにおけるrFVIIaの有効性評価では著効64.9%，有効4.7%，やや有効19.2%，無効11.2%で，その有効率は69.6%であった．このうち「rFVIIa単独治療群」に限ると著効60.2%，有効5.5%で有効率は65.7%であった．一方，「rFVIIaと他剤併用治療群」では著効69.2%，有効4.0%で有効率は73.1%であった（**Table 1**）．「rFVIIa単独治療群」と「rFVIIaと他剤併用治療群」を併せて，疾患別に有効率を求めた結果では血友病Aが71.4%（623/872出血エピソード），血友病Bが64.9%（122/188出血エピソード），後天性血友病Aが53.3%（24/45出血エピソード）で後天性血友病Aで有効率が低かった．

「rFVIIa単独治療群」528出血エピソードにおけるカテゴリー別解析結果は，**Table 2a**に示したごとく出血部位，初回投与がなされた場所（自宅あるいは病院），初回投与量，平均投与間隔および投与回数に有意差が認められた．すなわち，初回投与がなされた場所に関しては，病院治療に比べ在宅治療群で有意に有効率が高く，投与回数については3回以内では70%以上の高い有効率を示したが，それより多い回数では有効率が低かった．また，平均投与間隔については3時間以内で71.4%の有効率を示したのに対し，3時間を超えた場合の有効率は39.4%であった．

出血から治療開始までの時間は，先天性血友病では在宅治療群で平均2.9時間（SD；3.9時間，範囲；0～44.5時間，中央値；2.0時間），病院治療群で平均12.3時間（SD；33.6時間，範囲；0～367.8時間，中央値；3.0時間），一方，後天性血友病Aの初回出血エピソードで平均5日（SD；8日，範囲；2.5時間～36日，中央値；2日）であり，後天性血友病A患者の初回出血エピソードにおいて著しく長かった．

「rFVIIaと他剤併用治療群」の577出血エピソードについて，カテゴリー別に解析した結果（**Table 2b**）は，「rFVIIa単独治療群」の528出血エピソード（**Table 2a**）とほぼ同様の成績であった．

「推奨治療条件群（112出血エピソード）」および「非推奨治療条件群（170出血エピソード）」の有効率の比較では，併用治療の有無に関わらず前者の有効率が有意に高かった（オッズ比；2.42，p=0.0020およびオッズ比；5.83，$p < 0.0001$）（**Table 3a**および**3b**）．これらの群で，3つの要件のうち平均投与間隔が有効率の差に最も大きく寄与しており次いで初回投与量の順で，いずれも統計学的に有意であった．しかし，出血から初回投与までの時間を3時間で区切った場合の寄与は統計学的に有意ではなかった（**Table 4**）．

1,105出血エピソードのうち，消化管出血17エピソード，頭蓋内出血7エピソードでrFVIIaが使用された．有効率は消化管出血で41.2%（7/17出血エピソード），頭蓋内出血で85.7%（6/7出血エピソード）であった．投与回数は，消化管出血で1～117回，頭蓋内出血で5～122回であった．投与間隔をみると，投与早期は2～3時間ごとで，その後徐々に4～12時間に延長された．

3．安全性

102例中20例で報告された副作用42件中30件（71%）は臨床検査値異常であった（**Table 5**）．臨床検査値異常を除く12件のうち，中心静脈カテーテル閉塞，視野異常，脳梗塞の疑いおよび間質性肺炎の3例4件が重篤な副作用であった．これらの重篤な副作用はいずれも軽快または回復した（**Table 6**）．また，rFVIIa投与との明らかな関連性は認められなかった．

本剤の重篤な副作用の発現症例率は，インヒビターを保有する先天性および後天性血友病で，それぞれ2.7%（2例/75例），4.3%（1例/23例）で統計学的な有意差は認められなかった．本剤の総出血エピソードに占める重篤な副作用発現率は，先天性および後天性血友病で，それぞれ0.2%（3件/1,383出血エピソード），

Table 1 Comparison of efficacy rate between "rFVIIa group" and "rFVIIa + concomitant medications group"

	Excellent	Good	Partial	Poor	Total	Efficacy rate,% (effective*/total episodes)
rFVIIa	318	29	95	86	528	65.7%
	(60.2%)	(5.5%)	(18.0%)	(16.3%)	(100.0%)	(347/528)
rFVIIa + concomitant medications †	399	23	117	38	577	73.1%
	(69.2%)	(4.0%)	(20.3%)	(6.6%)	(100.0%)	(422/577)
Total	717	52	212	124	1,105	69.6%
	(64.9%)	(4.7%)	(19.2%)	(11.2%)	(100.0%)	(769/1,105)

* effective:'Excellent' and 'Good'

† concomitant medications: coagulation factor products, non-steroidal anti-inflammatory drugs, steroids,and/or tranexamic acid

Table 2a Efficacy rate according to investigated variables (rFVIIa group)

	No. of effective† /total episodes	Efficacy rate (%)	p-value χ^2 test
Type of bleeding			
Haemarthrosis	242 / 366	66.1	
Intramuscular bleeding	41 / 62	66.1	
Subcutaneous bleeding	27 / 41	65.9	
Intraoral bleeding	11 / 15	73.3	
Open bleeding	10 / 12	83.3	p=0.013*
Haematuria	2 / 12	16.7	
Epistaxis	8 / 9	88.9	
Gastrointestinal bleeding	4 / 8	50.0	
Intracranial bleeding	2 / 2	100.0	
Haemophilic pseudotumor	0 / 1	0.0	
Severity of bleeding symptom			
Mild	107 / 165	64.8	
Moderate	179 / 259	69.1	p=0.159
Severe	61 / 104	58.7	
Treatment status at initial dosing			
At home	188 / 264	71.2	p=0.010*
At hospital	159 / 264	60.2	
Initial dose, μg/kg			
<90	85 / 145	58.6	p=0.042 *
90≤	261 / 381	68.5	
Unknown	1 / 2	50.0	
Time from the onset of haemorrhage to initial dosing, hours			
≥3	187 / 259	72.2	p=0.500
3<	100 / 146	68.5	
Unknown	60 / 123	48.8	
Mean dosing interval, hours ‡			
≥3	232 / 325	71.4	p<0.001**
3<	41 / 104	39.4	
Unknown	0 / 6	0.0	
No. of doses			
1	74 / 93	79.6	
2	122 / 155	78.7	
3	86 / 116	74.1	p<0.001**
4-6	48 / 94	51.1	
7-	17 / 69	24.6	
Unknown	0 / 1	0.0	
Total	347 / 528	65.7	

† effective:'Excellent' and 'Good'

‡ Mean dosing interval, hours: The episodes of the single dose are excluded.

Table 2b Efficacy rate according to investigated variables (rFVIIa + concomitant medications group†)

	No. of effective‡ /total episodes	Efficacy rate (%)	p-value χ^2 test
Type of bleeding			
Haemarthrosis	310 / 392	79.1	
Intramuscular bleeding	54 / 82	65.9	
Subcutaneous bleeding	18 / 27	66.7	
Intraoral bleeding	14 / 24	58.3	
Open bleeding	7 / 11	63.6	
Haematuria	0 / 9	0.0	
Epistaxis	9 / 10	90.0	p<0.001**
Gastrointestinal bleeding	3 / 9	33.3	
Intracranial bleeding	4 / 5	80.0	
Haemophilic pseudotumor	0 / 1	0.0	
Intraperitoneal bleeding	1 / 2	50.0	
Respiratory system bleeding	1 / 1	100.0	
Others	1 / 3	33.3	
Unknown	0 / 1	0.0	
Severity of bleeding symptom			
Mild	124 / 158	78.5	
Moderate	224 / 303	73.9	p=0.020*
Severe	71 / 112	63.4	
Unknown	3 / 4	75.0	
Treatment status at initial dosing			
At home	301 / 357	84.3	p<0.001**
At hospital	121 / 220	55.0	
Initial dose, μg/kg			
<90	27 / 64	42.2	p<0.001**
90≤	392 / 507	77.3	
Unknown	3 / 6	50.0	
Time from the onset of haemorrhage to initial dosing, hours			
≥3	233 / 312	74.7	p=0.792
3<	153 / 201	76.1	
Unknown	36 / 64	56.3	
Mean dosing interval, hours#			
≥3	137 / 211	64.9	p<0.001**
3<	33 / 96	34.4	
Unknown	6 / 10	60.0	
No. of doses			
1	246 / 260	94.6	
2	73 / 96	76.0	
3	44 / 62	71.0	p<0.001**
4-6	31 / 77	40.3	
7-	25 / 79	31.6	
Unknown	3 / 3	100.0	
Total	422 / 577	73.1	

† concomitant medications: coagulation factor products, non-steroidal anti-inflammatory drugs, steroids, and/or tranexamic acid

‡ effective:'Excellent' and 'Good'

Mean dosing interval, hours: The episodes of the single dose are excluded.

Table 3a Comparison of efficacy between 'recommended practice group*' and 'non-optimal practice group' (rFVIIa group)

	Recommended practice group	Non-optimal practice group	Odds ratio (95%CI) †
Effective	90 (80.4%)	106 (62.4%)	2.42 (1.38 - 4.24)
Ineffective	22 (19.6%)	64 (37.6%)	p=0.0020
Total	112 (100.0%)	170 (100.0%)	

* recommended practice group:a group meeting all 3 requirements for correct use (specified as "initial dose≥90 μg/kg", "time from the onset of haemorrhage to initial dose≤3 hours" and "mean dosing interval≤3 hours")

† logistic regression analysis

Table 3b Comparison of efficacy between 'recommended practice group*' and 'non-optimal practice group' (rFVIIa + concomitant medications group†)

	Recommended practice group	Non-optimal practice group	Odds ratio (95%CI) ‡
Effective	42 (82.4%)	36 (44.4%)	5.83 (2.51 - 13.6)
Ineffective	9 (17.6%)	45 (55.6%)	p <0.0001
Total	51 (100.0%)	81 (100.0%)	

* recommended practice group:a group meeting all 3 requirements for correct use (specified as "initial dose≥90 μg/kg", "time from the onset of haemorrhage to initial dose≤3 hours" and "mean dosing interval≤3 hours")

† concomitant medications:non-steroidal anti-inflammatory drugs, steroids, and/or tranexamic acid (excluding coagulation factor products)

‡ logistic regression analysis

Table 4 Consideration of factors affecting efficacy

		Odds ratio (95%CI) *
Initial dose, μg/kg	90 ≤	1.89 (1.17 - 3.04)
	< 90	p=0.0090
Time from the onset of haemorrahage to initial dosing, hours	≤ 3	1.44 (0.91 - 2.28)
	3 <	p=0.1232
Mean dosing interval, hours	≤ 3	3.05 (1.89 - 4.93)
	3 <	p<0.0001

*logistic regression analysis: explaining variable are initial dose, time from haemorrahage to initial dosing and mean dosing intervals

Table 5 Number of adverse drug reactions

Adverse drug reaction	No. of events from Post Marketing Study	No. of events from Spontaneous report
Laboratory findings		
AST increased	1	
ALT increased	3	
Serum alkaline phosphatase increased	2	
LDH increased	3	
Serum bilirubin increased	2	
White blood cell count increased	1	
White blood cell count decreased	1	
D-dimer increased	4	
Thrombin-antithrombin complex increased	2	
Serum FDP increased	3	
Plasma fibrinogen increased	2	
Elevated PT (prothrombin activity index)	3	
Microscopic haematuria	1	
Blood pressure increased	2	
Blood pressure decreased		1 *
Symptoms		
Palpitations	2	
Nausea	1	
Pain	1	
Fever	1	2
Dull headache	1	
Headache	1	
Flushed face	1	
Abdominal pain		1
Complications		
Urticaria		1
Central venous catheter occlusion	1 *	
Suspected cerebral infarction	1 *	1 *
Abnormality in visual field (Haemianopia)	1 *	
Interstitial pneumonia	1 *	
Acute renal failure		1 *
Total	42	7

*Serious adverse reaction

1.3% (1件/78出血エピソード)，また投与回数に占める重篤な副作用の発現率も，それぞれ0.06% (3件/4,643回)，0.3% (1件/394回) と，いずれの場合も先天性および後天性血友病間で統計学的な有意差は認められなかった．

さらに，本調査とは別に7件の副作用が医療関係者から自発的に報告された．このうち脳梗塞の疑い，急性腎不全および血圧低下の3件が重篤な副作用と判定された (**Table 6**)．自発報告における重篤な副作用のいずれもrFVIIa投与との明らかな関連性は認められなかった．

考 察

本調査は，既に著者らによって報告したインヒビターを保有する先天性重症血友病Aおよび血友病B患者のrFVIIa治療に関する臨床試験成績 (58%)[6] とほぼ同様の有効率 (「rFVIIa単独治療群」65.7%) を示した．さらに，「著効」と評価された割合は，臨床試験成績 (31.2%)[6]

のほぼ2倍（60.2%）であった．これらより，rFVIIaの市販後の日常診療における有効性が確認された．

最近Parameswaranらが報告した米国のHemophilia and Thrombosis Research Society Registry（HTRS）の結果[7]では，先天性血友病患者の出血に対するrFVIIaの有効率（87%）は，本調査の結果と比較して高かった．この違いは，試験構成や治療環境の相違によるものと思われる．まず，本調査では有効性は初回治療後12時間以内に評価されたが，Parameswaranらの報告では72時間の時点で評価されている．一般的には，再出血を認めなければ，治療開始後の時間経過に従って臨床症状の改善は明確になるので，評価ポイントが遅いほど有効性の評価は高まることが推測される．また，米国と日本では，出血発現から治療開始までの時間の差があると思われる．その理由として初回投与の場所（病院か自宅か）の違いが考えられる．Parameswaranらの報告では初回治療の88%が自宅で行われていたのに対し，本調査では50%に過ぎなかった．本調査では初回投与が自宅で行われた群では病院で行われた群に比べ止血効果が高かったが，これは出血から治療開始までの時間が在宅治療ではより短かったためと思われる．先天性血友病患者については，出血発現から治療開始までの時間は在宅治療では平均2.9時間であり，これに対して病院での治療では平均12.3時間であった．

インヒビターを保有する先天性血友病患者を対象として行われた軽度～中等度出血エピソードの在宅治療に関する米国での1年間の試験[8]でも，早期治療の重要性が明らかにされている．この試験では88%の症例が有効と判定され，それらの症例は出血発現から平均1.6時間（SD；3.2時間）でrFVIIaの投与が開始されていた．わが国で在宅自己注射療法を行っている患者は血友病Aで58%，血友病Bで44%[9]と海外に比較して低い．血友病の止血管理では，インヒビターの有無にかかわらず，出血早期の治療開始が重要である．患者のQOLの向上や社会適応を広げるという面からも在宅自己注射療法の条件[10]が整っている患者には積極的に家庭療法を導入することが望ましい．

また，「rFVIIa単独治療群」の「推奨治療条件群」では「非推奨治療条件群」に比べ有効率が有意に高かったことから，推奨治療条件を満たす使用法により，本剤の治療効果をさらに改善することが期待できる．本調査ではカテゴリー別解析「出血から初回投与までの時間」については統計学的な有意差が認められず，その要因は不明であるが，出血発生後の早期治療開始は一般的に重要と考えられる．本調査では推奨条件を満たした治療エピソードは全体の40%に過ぎなかったことから，これらの条件（「初回投与量が90 μg/kg以上」，「出血から初回投与までの時間が3時間以内」および「平均投与間隔が3時間以内」）の重要性について，患者，介護者および医療者に対して再確認する必要がある．

後天性血友病A患者の出血エピソードでは，先天性血友病患者に比べ本剤の有効率が低かった（53.3% vs. 70.3%）．これは，インヒビターを有する血友病という点では類似するものの，診断と治療をめぐる環境がこの患者集団間で異なるためと考えられる．通常，先天性血友病がインヒビターを発生した際の診断および治療は血友病専門医によって行われる．一方，後天性血友病の初診診療科は多岐にわたり，血液内科などの専門医への紹介から確定診断までに時間を要することが多い．そのため適切な止血治療開始まで時間がかかる傾向にあり，しばしば止血管理に難渋する．出血から治療開始までの時間は，後天性血友病患者の初回出血エピソードでは，先天性血友病症例に比較し明らかに長時間を要していた．後天性血友病の止血治療効果を改善するためには，本疾患の早期診断・早期治療に関する一般医師への理解を広める必要がある．

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Table 6 Serious adverse reactions in haemophilia patients with inhibitors under rFVIIa treatment

Sex	Age, Year	Type of disease	Concomitant disease	Adverse drug reaction	Time of onset (Time from the final dose)	Outcome	Causal relationship with rFVIIa *	Clinical course
from Post Marketing Study								
Male	3	Congenital haemophilia A	Porencephaly Iron-deficiency anemia	Central venous catheter occlusion	3 weeks later	Remitted	Impossible to assess	After administration of rFVIIa, the patient received prothrombin complex concentrate 4 times. Catheter was removed as completely occluded. The indwelling period of catheter was unknown.
Male	38	Congenital haemophilia A	Epilepsy Chronic hepatitis C HIV	Abnormality in visual field (Haemianopia)	1 day later	Recovered	Possible	The patient had a convulsive seizure and admitted to the hospital on Jul 29. Although a brain CT showed negative image, the doctor administered rFVIIa to the patient in consideration of the possibility of cerebral haemorrhage that could not be detected by the image in the acute phase. An electroencephalogram of Aug 2 revealed epilepsy waves. He was initiated Aleviatin (phenytoin sodium) from Aug 5. During the period of Jul 30 to Sep 3, he was given a total of 26 bolus injections of rFVIIa because of recurrent intra-articular bleedings in the right knee, the gastrointestinal bleeding and intra-articular bleeding in the right elbow joint. During his hospitalization, he had also complained the lowering of attentiveness and slowness of thinking. On Sep 4 he was discharged as no evidence of epilepsy was present. On Sep 5 of his discharge, he complained right haemianopia. However, a perimetry of Sep 12 showed no signs of haemianopia. A brain MRI of Oct 2 showed high signal regions in the right frontal lobe, right putamen and right anterior horn, indicating cerebral infarction. During the month his subjective symptoms of haemianopia and lowering of attentiveness subsided. A brain CT of Oct 30 showed low density area in the right frontal lobe. However, in the follow up brain MRI of Nov 27 all the high signal findings previously noted were disappeared.
				Suspected cerebral infarction	1 month later	Recovered	Possible	
Male	70	Acquired haemophilia A	Atrial fibrillation	Interstitial pneumonia	3 months later	Remitted	Impossible to assess	Chest X-ray films disclosed bilateral interstitial changes and a diagnosis of interstitial pneumonia was made. The patient was hospitalized and received steroid pulse therapy. After remission was achieved, the patient was discharged.

Sex	Age, Year	Type of disease	Concomitant disease	Adverse drug reaction	Time of onset (Time from the final dose)	Outcome	Causal relationship with rFVIIa *	Clinical course
from Spontaneous report								
Male	73	Acquired haemophilia A	Hemolytic anemia Chronic renal failure Diabetes mellitus	Suspected cerebral infarction	1 day later	Fatal	Possible	The patient had a history of hypertension, cerebral infarction, hyperlipidemia, and gastric cancer. rFVIIa was administered to treat haemorrhage at the site of a catheter for IVH. Mandibular respiration, loss of consciousness during conversation, and quadriplegia occurred at 17.5 hours after administration. The patient died on the same day. A confirmed diagnosis using image analysis was not made.
Male	28	Congenital haemophilia A	Chronic Hepatitis C Hyperuricemia Hepatitis B virus carrier	Acute renal failure	Under treatment	Recovered	Possible	rFVIIa was administered to treat haematuria and gingival bleeding. Since anuria was persistent, a urethral catheter was inserted, but the urine volume was only about 20 mL. Blood tests revealed renal failure (BUN: 60, Cr: 4.99). On the next day, anuria continued with worsening renal failure (BUN: 75, Cr: 7.89), so dialysis was instituted. Diuresis occurred and the two parameters returned to normal after 9 days.
Male	76	Acquired haemophilia A	Prostate cancer	Hypotension	4 days later	Fatal	Possible	rFVIIa was administered to treat gingival bleeding. The blood pressure decreased to 60-70 mmHg at 4 days after administration of rFVIIa. It did not respond to blood transfusion, intravenous infusion, or vasopressors. The patient died at 6 days after administration of rFVIIa.

* Causal relationship with rFVIIa:Reporter's causality