

Fig. 5 Adverse effects from the treatment with bypassing agents

Six adverse events were reported in five centers. An event of acute myocardial infarction, two events of DIC, and two events of changes in laboratory data for DIC markers resulted from the treatment with aPCC, whereas an event of nausea and headache resulted from the treatment with PCC. In contrast, no adverse events were observed with rFVIIa treatment. From their comments, the event of DIC and the changes in laboratory data for DIC markers might be caused by massive use and prolonged use of bypassing agents, respectively.

aPCC : activated prothrombin complex concentrates, PCC : prothrombin complex concentrates, rFVIIa : recombinant activated factor VII, DIC : disseminated intravascular coagulation

い¹²⁾。アンケート結果では活性化部分トロンボプラスチン時間 (activated partial thromboplastin time ; aPTT) を用いる施設が5施設, プロトロンビン時間 (prothrombin time ; PT), トロンボエラストグラフィ (thromboelastography ; TEG) を用いる施設がそれぞれ4施設, rFVIIa製剤の使用時は第VII因子活性を測定する施設が2施設あった。しかし, これらの検査は中和療法時の第VIII (IX) 因子活性によるモニタリングと比べると, 止血効果を適正に反映したものとはいえない。一部の施設で行われている凝固波形解析やトロンビン生成試験はより確実に止血状況を反映する検査として期待されているが, 一般的ではない上に方法論もまだ確立されたものがない (Fig. 4)。

9. バイパス製剤に関する副作用の経験

バイパス止血療法の副作用は5施設から6

件の回答があった。このうち, DIC2件, DICマーカーの変動2件および心筋梗塞1件はaPCC使用例であり, 嘔気・頭痛の1件はPCC使用例であった。なお, DICおよびDICマーカーの変動の各1件はそれぞれaPCCの大量投与もしくは長期使用時のものであった。一方, rFVIIa製剤に関する副作用の回答はなかった (Fig. 5)。

考 案

海外におけるインヒビター止血療法のガイドラインのうち, カナダの The Association of Hemophilia Clinic Directors of Canada (AHCDC)⁶⁾, イタリアの The Italian Association of Haemophilia Centres (AICE)⁹⁾, 英国の The UK Haemophilia Centre Doctors' Organization (UKHCDO)¹⁰⁾ およびオーストラリアの Australian Health Ministers' Advisory Council

(AHMAC)¹¹⁾の各ガイドラインに記載されている製剤選択を Table 5 にまとめた。それによると5BU/ml未満のローレスポンダーではすべてのガイドラインでHD FVIII (IX) が第一選択であった。これは、ローレスポンダーでは第VIII (IX) 因子製剤を繰り返し投与しても、5BU/mlを越えるインヒビターの上昇が見られないことから、HD FVIII (IX) による中和療法がより効果的かつ経済的であるためと思われる。一方、今回のアンケート結果ではローレスポンダーの軽度出血の際にはHD FVIII (IX) のみならず、バイパス製剤を選択する施設が少なくなかった。この理由として、わが国ではいまだに低力価と高力価インヒビターの境界が5BU/mlか10BU/mlかで混乱しており、後者ではHD FVIII (IX) の投与量が大量になること、さらに、その後インヒビターが中和困難な力価まで上昇するのではないかと危惧されることなどが挙げられる。また、中和療法を行うためには最新のインヒビター値が必要になるが、バイパス止血療法ではそれが不要なく、比較的容易に投与できるなどの理由が考えられる。

現在の力価が5BU/ml未満のハイレスポンダー患者の軽度出血の際には、海外のガイドラインではいずれもバイパス製剤が第一選択であった⁹⁻¹¹⁾。同様に、今回のアンケート結果でもバイパス製剤が最も多く選択されていた。これは将来に起こるかもしれない重篤な出血や大手術に備えてHD FVIII (IX) による治療の選択肢を残しておくためと考えられる。一方、現在5BU/ml未満のハイレスポンダーの重度出血もしくは手術の場合は、オーストラリアのガイドライン¹¹⁾ではバイパス製剤を、その他の国ではいずれもHD FVIII (IX) を第一選択としていた^{9,10)}。今回のアンケート結果でも同様にHD FVIII (IX) とバイパス製剤で選択が分かれていた。この場合の製剤選択は出血の重症度や手術の内容、すなわち生命にかかわる出血や手術であるかどうかのポイントになるとと思われる。海外のガイドラインではlife-threatening

もしくはlimb-threateningといった表現がされているが、こういった場合には、HD FVIII (IX) の投与により既往免疫反応が起こるまでの4-7日間、第VIII (IX) 因子活性でモニタリングしながら確実に止血を図る方法が現時点ではベストオプションであると思われる。しかし、そのためには直近のインヒビター値が判明していること、モニタリングがリアルタイムに行われることが必要であり、このような治療は検査体制の整った経験豊富な施設で行われるべきである。

現在5BU/mlを超えるハイレスポンダーではすべての海外のガイドラインでバイパス製剤が第一選択であった⁹⁻¹¹⁾。バイパス製剤の止血効果が不十分なときは血漿交換や免疫吸着カラムを用いてインヒビター力価を低下させた上でHD FVIII (IX) による中和療法が行われる。今回のアンケートでも10BU/ml超ではバイパス製剤のみが選択されていたが、5-10BU/mlの重度出血もしくは手術時ではHD FVIII (IX) を選択する施設が3施設あった。これは、近年、第VIII (IX) 因子製剤の安全性が向上するとともに濃縮率が高くなり、循環量を過剰にすることなくインヒビターの中和が可能になったためと思われる。ただし、5-10BU/mlでのインヒビターの中和については、海外のガイドラインでは全く選択肢が上がっていない。

次にバイパス製剤の選択、すなわち、rFVIIa製剤とaPCCの選択についてであるが、今回のアンケート結果をみるとハイレスポンダー症例ではあえて優先順位をつけないという施設が最も多く、5施設あった。次に、rFVIIa製剤を優先して使用する施設が2施設、インヒビターが5もしくは10BU/ml未満の時のみrFVIIa製剤を優先して選択すると回答した施設がそれぞれ1施設ずつあった。一方、ローレスポンダーでもバイパス製剤を選択肢の一つとする施設が8施設あった。このうち、両者に優先順位を付けない施設が4施設、rFVIIa製剤を優先する施設が3施設、出血時はrFVIIa製剤を優先する

Table 5 Treatment option for bleeding or surgery in patients with hemophilia and inhibitors on the overseas guidelines

	High responder					
	Low responder			>5 BU/ml		
	<5 BU/ml		<5 BU/ml		>5 BU/ml	
	Mild bleeding	Severe bleeding/Surgery	Mild bleeding	Severe bleeding/Surgery	Mild bleeding	Severe bleeding/Surgery
AHCDC Canada 2000 ⁹⁾	HD FVIII	HD FVIII	1. Bypassing agents 2. Porcine FVIII	1. HD FVIII/Porcine FVIII 2. Bypassing agents	1. Bypassing agents 2. Porcine FVIII	1. Bypassing agents/Porcine FVIII 2. HD FVIII after plasma exchange/immunoabsorption
AICE Italy 2005 ³⁾	1. HD FVIII (IX) 2. Bypassing agents	1. HD FVIII (IX) 2. Bypassing agents	1. Bypassing agents 2. HD FVIII (IX)	1. HD FVIII (IX) 2. Bypassing agents	Bypassing agents	1. Bypassing agents 2. HD FVIII (IX) after immunoabsorption
UKHDCO UK 2006 ¹⁰⁾	1. HD FVIII (IX) 2. Bypassing agents	1. HD FVIII (IX) 2. Bypassing agents	Bypassing agents	1. HD FVIII (IX) 2. Bypassing agents	Bypassing agents	1. Bypassing agents 2. HD FVIII (IX) after plasma exchange/immunoabsorption
AHMAC Australia 2006 ¹¹⁾	HD FVIII	HD FVIII	Bypassing agents	Bypassing agents	Bypassing agents	1. Bypassing agents 2. HD FVIII after plasma exchange/immunoabsorption

AHCDC : The Association of Hemophilia Clinic Directors of Canada, AICE : The Italian Association of Haemophilia Centres
 UKHDCO : The UK Haemophilia Centre Doctors' Organisation, AHMAC : Australian Health Ministers' Advisory Council
 HD FVIII (IX) : high doses of factor VIII (IX) concentrates, BU : Bethesda unit

が、手術時は優先順位を付けない施設が1施設であった。その他コメントとして、過去の出血時の投与経験から効果が高いと考えられるものを選択するというものや出血から輸注までの時間が短い場合は rFVIIa 製剤を選択し、長い場合は aPCC を選択するというものがあった。なお、aPCC には第 VIII 因子のフラグメント、特に、C2 フラグメントの残存が証明されており、これが aPCC 使用による既往免疫反応の惹起につながり得ることも治療の選択上考慮されている可能性がある¹³⁾。海外のガイドラインをみると、オーストラリア以外の国では両者の優劣をつけていない⁹⁾⁻¹¹⁾。実際、関節内出血に対する両者の有効性を比較した最近の研究 (FENOC (Feiba NovoSeven Comparative) study) では、治療 12 時間間までは一部で有効性に差が見られたものの、概ね同等の有効性を有すると報告されている¹⁴⁾。

今回、わが国のインヒビター止血療法、とくにバイパス療法について、その現状が明らかとなった。このうち、治療製剤の選択やバイパス製剤の使用方法の一部で意見が分かれる点が見られた。今後、科学的なエビデンスの蓄積により、バイパス止血療法の国際的標準化が進むことが期待される一方、わが国でもガイドライン作成に向けて、これらの意見を集約していく必要があると思われる。しかし、わが国の保険診療上 aPCC の使用に一部制限が加わっているという特殊な事情のため、海外のガイドラインと同じ条件では議論できない部分があり、このような制約の早期撤廃が望まれるところである。

文 献

- 1) McMillan CW, Shapiro SS, Whitehurst D, Hoyer LW, Rao AV, Lazerson J : The natural history of factor VIII : C inhibitors in patients with hemophilia A : a national cooperative study. *Blood* 71 : 344-348, 1988.
- 2) Gill FM : The natural history of factor VIII inhibitors in patients with hemophilia A. *Prog Clin Biol Res* 150 : 19-29, 1984.
- 3) Katz J : Prevalence of FIX inhibitors among patients with hemophilia B : results of large scale North American survey. *Haemophilia* 2 : 31-38, 1996.
- 4) Warrior I : ITI in hemophilia B : Possibilities and problems. *International Monitor of Haemophilia* 8 : 3-6, 2000.
- 5) White GC 2nd, Rosendaal F, Aledort LM, Lusher JM, Rothschild C, Ingerslev J : Factor VIII and Factor IX Subcommittee : Definitions in hemophilia. Recommendation of the scientific subcommittee on factor VIII and factor IX of the scientific and standardization committee of the International Society on Thrombosis and Haemostasis. *Thromb Haemost* 85 : 560, 2001.
- 6) The Association of Hemophilia Clinic Directors of Canada (AHCDC) : Suggestions for the management of factor VIII inhibitors. *Haemophilia* 6 : 52-59, 2000.
- 7) Schulman S : Safety, efficacy and lessons from continuous infusion with rFVIIa. rFVIIa-CI Group. *Haemophilia* 4 : 564-567, 1998.
- 8) Ludlam CA, Smith MP, Morfini M, Gringeri A, Santagostino E, Savidge GF : A prospective study of recombinant activated factor VII administered by continuous infusion to inhibitor patients undergoing elective major orthopaedic surgery : a pharmacokinetic and efficacy evaluation. *Br J Haematol* 120 : 808-813, 2003.
- 9) The Italian Association of Haemophilia (AICE) : Italian guidelines for the diagnosis and treatment of patients with haemophilia and inhibitors. *Haemophilia* 11 : 611-619, 2005.
- 10) The UK Haemophilia Centre Doctors' Organization (UKHCDO) : The diagnosis and management of factor VIII and IX inhibitors. *Br J Haematol* 133 : 591-605, 2006.
- 11) Australian Health Ministers' Advisory Council (AHMAC) : Evidence-based clinical practice guidelines for the use of recombinant and plasma-derived FVIII and FIX products. <http://www.nba.gov.au>, 2006.
- 12) Astermark J, Rocino A, Von Depka M, Van Den Berg HM, Gringeri A, Mautovani LG, Morado M, Garrido RP, Schiavoni M, Villar A, Windyga J : Current use of by-passing agents in Europe in the management of acute bleeds in patients with haemophilia and inhibitors. *Haemophilia* 13 : 38-45, 2007.
- 13) Yoshioka A, Kamisue S, Tanaka I, Kato M, Kohmura I, Shima M, Fukui H : Anamnestic response following infusion of prothrombin complex concentrates (PCC) and activated prothrombin complex concentrates (APCC) in hemophilia A patients with inhibitors. *Blood Coagul Fibrinolysis* 2 : 51-58, 1991.
- 14) Astermark J, Donfield SM, DiMichele DM, Gringeri A, Gilbert SA, Waters J, Berntorp E : A randomized comparison of bypassing agents in hemophilia complicated by an inhibitor. *Blood* 109 : 546-551, 2007.

1) McMillan CW, Shapiro SS, Whitehurst D, Hoyer LW, Rao AV, Lazerson J : The natural history of factor VIII : C inhibitors in patients with hemophilia A : a national cooperative study.

Current use of bypassing agents in Japan in patients with congenital hemophilia and inhibitors

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Key words: hemophilia, inhibitor, bypassing agents, high-dose FVIII (IX) concentrates

In order to elucidate current issues of hemostatic treatment using bypassing agents in patients with hemophilia and inhibitors, investigators from nine hemophilia centers in Japan were asked to complete a questionnaire. The questionnaire asked about the product choice for the management of mild bleeding, severe bleeding or surgery in low responders with a titer of <5 BU/ml, and high responders with a current titer of <5, 5-10, >10 BU/ml. They were also requested to report their experience with the usage of bypassing agents including off-label use as well as the monitoring tests and the adverse events. The survey revealed that the respondents were divided over the therapeutic choice for mild bleeding in low responders with a titer of <5 BU/ml and severe bleeding/surgery in high responders with a current titer of <5 BU/ml between the treatment with bypassing agents and the inhibitor neutralization with high doses of factor VIII (IX) concentrates. A consensus of the opinions would be needed for development of a domestic guideline for the treatment of patients with hemophilia and inhibitors.

Comparison Between Sendai Virus and Adenovirus Vectors to Transduce HIV-1 Genes Into Human Dendritic Cells

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Immuno-genotherapy using dendritic cells (DCs) can be applied to human immunodeficiency virus type 1 (HIV-1) infection. Sendai virus (SeV) has unique features such as cytoplasmic replication and high protein expression as a vector for genetic manipulation. In this study, we compared the efficiency of inducing green fluorescent protein (GFP) and HIV-1 gene expression in human monocyte-derived DCs between SeV and adenovirus (AdV). Human monocyte-derived DCs infected with SeV showed the maximum gene expression 24 hr after infection at a multiplicity of infection (MOI) of 2. Although SeV vector showed higher cytopathic effect on DCs than AdV, SeV vector induced maximum gene expression earlier and at much lower MOI. In terms of cell surface phenotype, both SeV and AdV vectors induced DC maturation. DCs infected with SeV as well as AdV elicited HIV-1 specific T-cell responses detected by interferon γ (IFN- γ) enzyme-linked immunospot (Elispot). Our data suggest that SeV could be one of the reliable vectors for immuno-genotherapy for HIV-1 infected patients. *J. Med. Virol.* 80:373–382, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: viral vector; immuno-genotherapy; AIDS

INTRODUCTION

Introduction of highly active anti-retroviral therapy (HAART) has improved the clinical course of patients infected with human immunodeficiency virus type 1 (HIV-1) dramatically. However, there are many obstacles to the long-term administration of anti-retroviral drugs, such as metabolic disorders, emergence of drug resistant viruses, and high medical expenses. The combination of therapeutic vaccines and HAART could not only reduce the adverse effects of HAART but also decrease the medical expenses especially in developing countries.

Cellular immune responses play a crucial role in controlling the replication of HIV-1 [Yang et al., 1997;

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Matano et al., 1998; Brander and Walker, 1999; Jin et al., 1999; McMichael and Rowland-Jones, 2001]. One of the possible strategies for treating HIV-1 infection is to enhance the cellular anti-viral capacity. Since dendritic cells (DCs) have high ability of antigen presentation, they have been used as stimulators of T-cell responses by inserting HIV-1 antigens [Engelmayer et al., 2001; Stubbs et al., 2001; Tsunetsugu-Yokota et al., 2003; Mwau et al., 2004].

Sendai virus (SeV), one of the members of *Paramyxoviridae*, is an enveloped virus with a nonsegmented negative-strand RNA genome. It causes severe respiratory disease in mice but is nonpathogenic for humans. SeV vector has been developed and shown to have high gene transduction efficiency and protein expression in different cell lineages [Kato et al., 1996; Kawana-Tachikawa et al., 2002]. In addition, the cytoplasmic replication of SeV precludes the integration of its genetic information into cellular genome. Recently, the second generation SeV vector deficient in F gene (dF-SeV) has been developed [Li et al., 2000]. The dF-SeV has been proved not to cause secondary infection. SeV and dF-SeV vectors could be promising systems to introduce HIV-1 genes into DCs for stimulating HIV-1 specific T-cell responses in primates and humans [Kano et al., 2002; Takeda et al., 2003; Kato et al., 2005].

Adenovirus (AdV), vaccinia virus, and retrovirus have been used as viral vectors for gene transfer into DCs [Engelmayer et al., 1999; Rea et al., 1999; Bonini et al., 2001; Rouas et al., 2002]. In this study, we compared the efficiency of inducing green fluorescent protein (GFP) and HIV-1 gene expression in human monocyte-derived DCs between AdV vectors and new viral vectors based on SeV. We showed that SeV vector transduced DCs efficiently and elicited HIV-1 specific T-cell responses. Our results suggest the potential use of SeV vector for immuno-genetherapy for HIV-1 infected patients.

MATERIALS AND METHODS

Viral Vectors

SeV carrying GFP (SeVGFP) [Agunprijono et al., 2000], HIV-1 NL4-3 Gag (SeVGag), and HIV-1 SF2 Env (SeVEnv) [Yu et al., 1997] were propagated in 10-day-old embryonated chicken eggs. The fusion protein (F)-defective SeV (dF-SeV) [Li et al., 2000] was propagated in the monkey kidney cell line expressing Sendai virus F gene product (LLC-MK2/F7) [Li et al., 2000] because the replication capacity of dF-SeV was incompetent. SeV particles were purified by 50% sucrose (w/v)/10 mM Tris-HCl and 30% sucrose (w/v)/10 mM Tris-HCl density centrifugation, dialyzed against Dulbecco's phosphate buffered saline (PBS) (Sigma, St. Louis, MO), and stored at -80°C . The titers of the vector stocks, determined on LLC-MK2 [Kato et al., 1996], were as follows; SeVGag: 5.2×10^8 CIU/ml, SeVEnv: 6.4×10^7 CIU/ml, SeVGFP: 5.2×10^8 CIU/ml, GFP/dF-SeV: 3.1×10^9 CIU/ml, SeV without inserts: 5.2×10^8 CIU/ml.

Recombinant AdV used in this experiment was derived from AdV type 5 and was replication-deficient

with deletion of E1 and E3 genes. The AdV carrying HIV-1 genes (*gag-pol*, *env*, *rev*, and RRE) was generated with AdV Expression Kit (TakaraBio, Shiga, Japan) according to the manufacturer's protocol. Each HIV-1 gene was inserted into the expression cassette of pAxCawt cosmid vector equipped with CAG promoter and rabbit beta-globin polyadenylation signal. All HIV-1 sequences were derived from SF2 strain [Levy et al., 1986]. 5' half and 3' half of *EcoRI* fragments from SF2 provirus were subcloned pUC19, generating pUC19-9B/R7 and pUC19-9B/R6, respectively. HIV-1 *gag-pol* gene was obtained from pUC19-9B/R7 after deleting the sequence upstream of *gag*. *NarI* site was introduced next to *gag* initiation codon by PCR using primers *gag/NarI-S* (5'-CAGGCGGCAAGGAGAGAGATGGGTGC-GAG-3') and *gag/ApaI-AS* (5'-CCTTTTCTAGGGG-CCCTGC-3') (restriction sites are underlined). PCR-amplified fragment was returned to *NarI* and *ApaI*-digested pUC19-9B/R7, generating pUC19-GP. The 4.5 kb *NarI-NdeI* fragment containing the HIV-1 *gag-pol* gene was inserted into the *SwaI* site of pAxCawt cosmid vector to create AdVGP. HIV-1 *rev* responsible element (RRE) was generated from pUC19-9B/R6 by PCR using primers *rev/pfIMI-S* (5'-GCCATAGAATG-GCCAAGGCAAAGAGAAGAGTGG-3') and *rev/BamHI-AS* (5'-GGGATCCCAAGGCACAGCAGTGGTTGC-3'). The PCR fragment was inserted between *pflMI* and *BamHI* site of pUC19-GP, and thus placed downstream of *gag-pol* gene. The consequent 4.9 kb *NarI-BamHI* fragment containing the HIV-1 *gag-pol*-RRE sequences was inserted into the *SwaI* site of pAxCawt cosmid vector to create AdVGP. HIV-1 *rev* gene was made by two-step PCR from *StuI-XhoI* fragment of pUC19-9B/R6. The 1st-PCR primer set was *rev/1st-S* (5'-CTCAGGACAGTCAGACTCATCAAGCTTCTCTATCAAAG-CAACCCGCTCC-3') and *rev-AS* (5'-GGCTATTCTT-TAGTTCCTGAATCCAATACTGCA-3'), and the 2nd-PCR primer set was *rev/2nd-S* (5'-GGATGGCAGGAA-GAAGCGGAGACAGCGACGAAGAGCTCCTCAGGACAG-3') and *rev-AS*. The PCR fragment was digested with *SphI* and *SpeI*, and inserted into the *SwaI* site of pAxCawt to create AdVRev. The absence of PCR errors was confirmed for all PCR-amplified fragments by sequencing. The 2.1 kb *MluI-XhoI* fragment of pUC19-9B/R6 containing the HIV-1 *env* gene was inserted into the *SwaI* site of pAxCawt cosmid vector to create AdVEnv. Recombinant AdV, Ax1w1 [Miyake et al., 1996] bearing no insert and AdVGFP [Miyake et al., 1996] expressing GFP, were kindly provided by Dr. Izumu Saito and Yumi Kanegae (University of Tokyo, Tokyo, Japan). AdV was propagated in 293 cells [Graham et al., 1977], purified by two rounds of CsCl density centrifugation [Kanegae et al., 1994], dialyzed against PBS containing 10% glycerol and stored at -80°C . The titer of the viral stocks was determined by a plaque-forming assay on 293 cells. The titers of each virus were as follows; AdVGPR: 7.6×10^9 pfu/ml, AdVEnv: 1.6×10^{11} pfu/ml, AdVRev: 2.7×10^{11} pfu/ml, AdVGFP: 5.9×10^{10} pfu/ml, Ax1w1: 4.7×10^{11} pfu/ml. Vector stocks were tested for the

absence of replication-competent AdV as described [Ishii-Watabe et al., 2003].

Cell Lines and Media

Monkey kidney cell line (LLC-MK2) was cultured in minimal essential medium (MEM) (Sigma) supplemented with 100 U of penicillin/ml, 100 U of streptomycin/ml, and 10% heat-inactivated fetal calf serum (FCS). Two hundred ninety three cells were cultured in Dulbecco's modified eagle medium (DMEM) (Sigma) supplemented with 100 U of penicillin/ml, 100 U of streptomycin/ml, and 10% FCS.

Generation of DCs

Immature myeloid DCs were generated from human peripheral blood mononuclear cells (PBMCs) using previously described methods [Nagayama et al., 2003]. Briefly, PBMCs of healthy adult volunteers were collected in heparinized tubes, subjected to density centrifugation over Ficoll-Paque Plus (Amersham Pharmacia Biotech, Piscataway, NJ), and washed twice with PBS. These PBMCs were plated on 10 cm PRIMARIA tissue culture dish (Becton Dickinson Labware, Franklin Lakes, NJ) and kept at 37°C for 30 min to remove nonadherent cells. Floating cells were removed gently by rinsing with 10 ml of PBS three times and the remaining adherent cells were cultured overnight in 6 ml of RPMI 1640 medium at 37°C. Cells were washed three times again on the next day with 10 ml of PBS and the remaining adherent cells were cultured for 7 days in DC medium [6 ml of RPMI 1640 supplemented with 100 U of penicillin/ml, 10 mg of streptomycin/ml, and 10% FCS, 300 ng of recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) (Wako, Osaka, Japan), and 300 ng of recombinant human interleukin-4 (rhIL-4) (Wako)]. After 7 days, DCs were collected with a scraper. Tumor necrosis factor- α (TNF- α) (Wako) was added to the DC medium at a final concentration of 50 ng/ml on day 7 in some experiments and cultured for another 2 days to generate mature DCs. The purity of DCs was >95% based on the expression of CD1a and CD11c and lack of expression of T-cell, B-cell, NK-cell, and monocyte lineage markers by flow cytometry (data not shown).

Infection of DCs With Viral Vectors

5×10^5 DCs were infected with SeV or AdV vectors for 1 hr at 37°C in a final volume of at least 500 μ l of serum-free RPMI 1640. After the infection, DCs were washed with serum-free RPMI 1640 medium and cultured in 24 well plates with 1 ml of the DC medium.

SDS-PAGE and Western Blot Analysis

DCs infected with SeV (SeVGag or SeVEnv) at a multiplicity of infection (MOI) of 2 or with AdV (AdVGFR, AdVEnv, AdVRev) at an MOI of 1,000 were harvested after 24 or 48 hr of infection, respectively. After washing with PBS, the cells were resuspended in

RIPA buffer (10 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1% NP-40, 0.1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 1 mM EDTA, complete mini as 1 \times protease inhibitor cocktail [Roche, Basel, Switzerland]), and kept on ice for 10 min. The suspension was spun for 5 min at 9,000g to remove cell debris. The amount of protein in the cell lysate was determined by protein assay kit (Bio-Rad Laboratories, Hercules, CA). Cell lysates containing 30 μ g of cellular protein were loaded onto a 10–20% Ready Gels J (Bio-Rad Laboratories) and electrophoretically transferred to immobilon polyvinylidene difluoride transfer membrane (Millipore, Billerica, MA). Western blot analysis was performed using Lumi-Light plus Western Blotting Kit (Roche) according to the manufacturer's instructions. Briefly, the membrane was blocked in 1% blocking solution at 4°C overnight. The membrane was incubated with mouse monoclonal antibodies against p24 (Advanced Biotechnologies, Inc., Columbia, MD) and gp120 (Immuno Diagnostics, Inc., Woburn, MA) of HIV-1 for 1 hr. The blots were then washed four times with 1 \times TBST and incubated with anti-mouse IgG conjugated with horseradish peroxidase (Roche). Proteins were illuminated by LumiLight Plus (Roche) and detected with Lumi Imager (Roche). Quantification was done by densitometric analysis with the Lumi Analyst software (Roche).

Immunostaining and Flow Cytometry

GFP expression and viability of DCs infected with SeVGFP, dF-SeVGFP, or AdVGFP were analyzed by flow cytometry. To determine the viability, 5×10^5 DCs in about 300 μ l of media were stained with 10 μ l of propidium iodide (PI) (50 μ g/ml; SIGMA). Events were acquired on a FACS-Caliber (Becton Dickinson) and analyzed with CellQuest software (Becton Dickinson) and Flow Jo software version 4.1 (Tree Star, Asland, OK).

To determine the effects of transduction on the expression of DC surface marker, immature DCs (imDCs) were cultured with the DC medium for 48 hr, mature DCs (mDCs) were cultured with the DC medium plus TNF- α for 48 hr. DCs transduced with SeV or AdV were cultured with the DC medium for 24 or 48 hr, respectively. Those DCs were stained with antibodies at 4°C for 20 min and then washed three times with PBS. Those cells were analyzed by flow cytometry after fixing 1% paraformaldehyde. The antibodies we used were as follows: fluorescein isothiocyanate-anti-Lineage (Lin-FITC) (CD3, CD14, CD16, CD19, CD20, CD56) (Becton Dickinson), phycoerythrin (PE)-anti-CD1a (Immunotech, Marseilles, France), PE-anti-CD83 (Immunotech), PE-anti-HLA-ABC (Dako), Peridinin chlorophyll protein (PerCP) -anti-CD4 (Becton Dickinson), PerCP-anti-HLA-DR (Becton Dickinson), allophycocyanin (APC)-anti-CD14 (Immunotech), APC-anti-CD40 (PharMingen), APC-anti-CD11c (PharMingen), Biotin-conjugated anti-CD86 (Becton Dickinson). Streptavidin-FITC (Becton

Dickinson) was employed as secondary reagents. FITC- (PharMingen, San Diego, CA), PE- (Dako Glostrup, Denmark), Per CP- (Becton Dickinson), APC- (Becton Dickinson) conjugated species- and isotype-matched, mAbs were used to determine the level of background staining.

Elispot Assay

We performed enzyme-linked immunospot (Elispot) assay to know the efficiency of HIV-1 specific T-cell induction by DCs infected with AdV or SeV vector. First, we developed mDCs as described above from PBMCs of two HIV-1-infected patients. On day 7, we infected those mDCs with AdV vector at an MOI of 1,000 or SeV vector at an MOI of 2 for 1 hr, or just added overlapping peptides (*gag*, *env*). We used the overlapping peptides derived from consensus B sequence since both patients were infected with subtype B HIV-1. We did not check the AdV sero-status of these two patients. Both patients were on HAART and have undetectable viral load (<50 copies/ml). CD4 counts of patients 1 and 2 are

408/ μ l and 336/ μ l, respectively. We used those mDCs as stimulators in Elispot assay. PBMCs from each patient were used as effectors cells. The protocol of Elispot assay was described previously [Furutsuki et al., 2004].

RESULTS

Sendai Viral Vectors Transduce DCs at Lower MOIs Than Adenoviral Vectors

We infected imDCs with SeVGFP, dF-SeVGFP or AdVGFP at different MOIs (Fig. 1) in order to know which MOI is the best for these three vectors. We stained these cells with PI to evaluate the expression of GFP in viable cells. In SeVGFP, the expression of GFP reached the maximum (32.5%) at an MOI of 2 and the mean fluorescent intensity (MFI) of GFP showed around 1,400 at all MOIs. However, as shown by the fraction of PI-positive cells, SeVGFP killed around 30% of DCs even at an MOI of 0.5. The staining pattern of DCs infected with dF-SeVGFP was similar to that of SeVGFP. In AdVGFP, both the percentage of GFP-positive cells and the MFI of

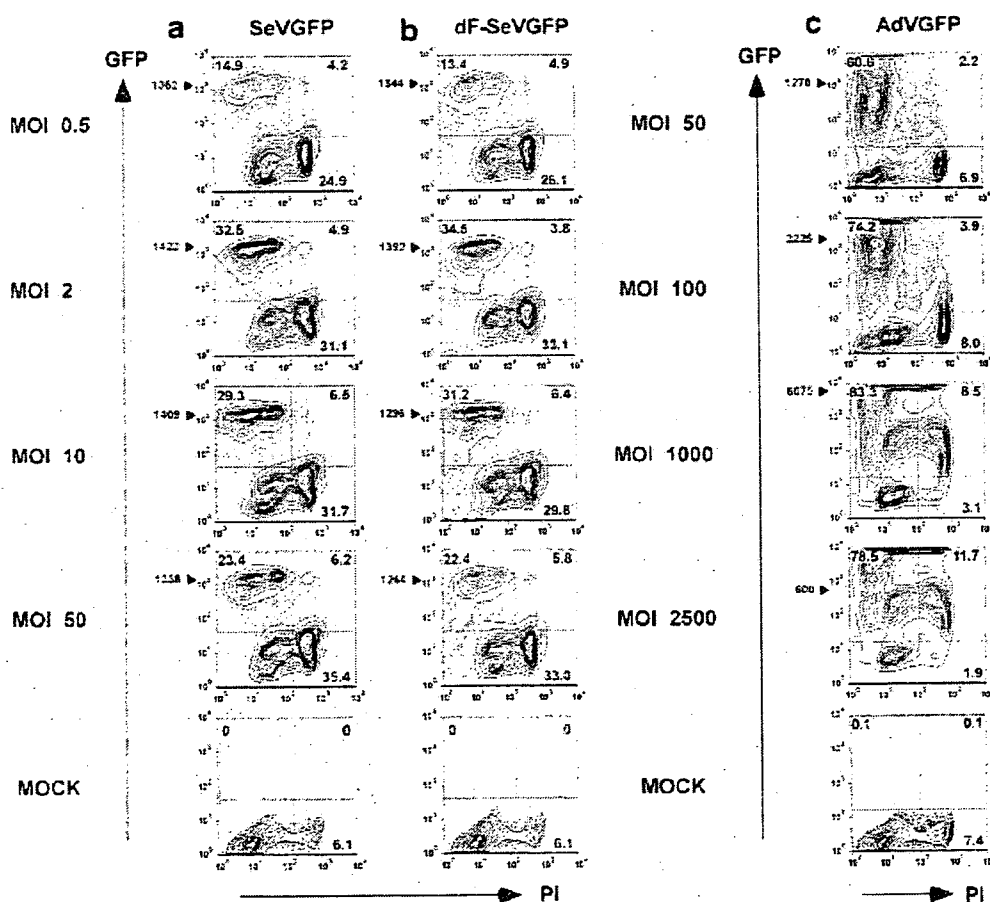


Fig. 1. Comparison of different MOIs for the maximum gene expression by SeV (a), dF-SeV (b), and AdV (c) vectors 48 hr after infection. Cell viability was determined by staining with PI. GFP expression and PI staining were analyzed by flow cytometry. The percentages of GFP- and PI-positive cells are shown on each corner. Arrowheads indicate MFI of GFP-positive cells within PI-negative fraction. The numbers in each panel represent the mean value of three independent experiments.

GFP increased up to an MOI of 1,000. Although PI-positive cells in AdV increased according to MOIs, the percentage of PI-positive cells was less than 14% even at the highest MOI: 2,500. From these results, SeV vector is likely to transduce DCs at much lower MOIs than AdV vector, but kill more DCs than AdV. We chose an MOI of 2 for SeVGFP and dF-SeVGFP, and an MOI of 1,000 for AdVGFP in the subsequent experiments.

Sendai Viral Vectors Showed Maximum Transduction Level Earlier Than Adenoviral Vectors

We next examined the time course of GFP expression (Fig. 2). We detected GFP-positive cells as early as 8 hr after infection in all three vectors. The proportion of GFP-positive cells reached the maximum level (around

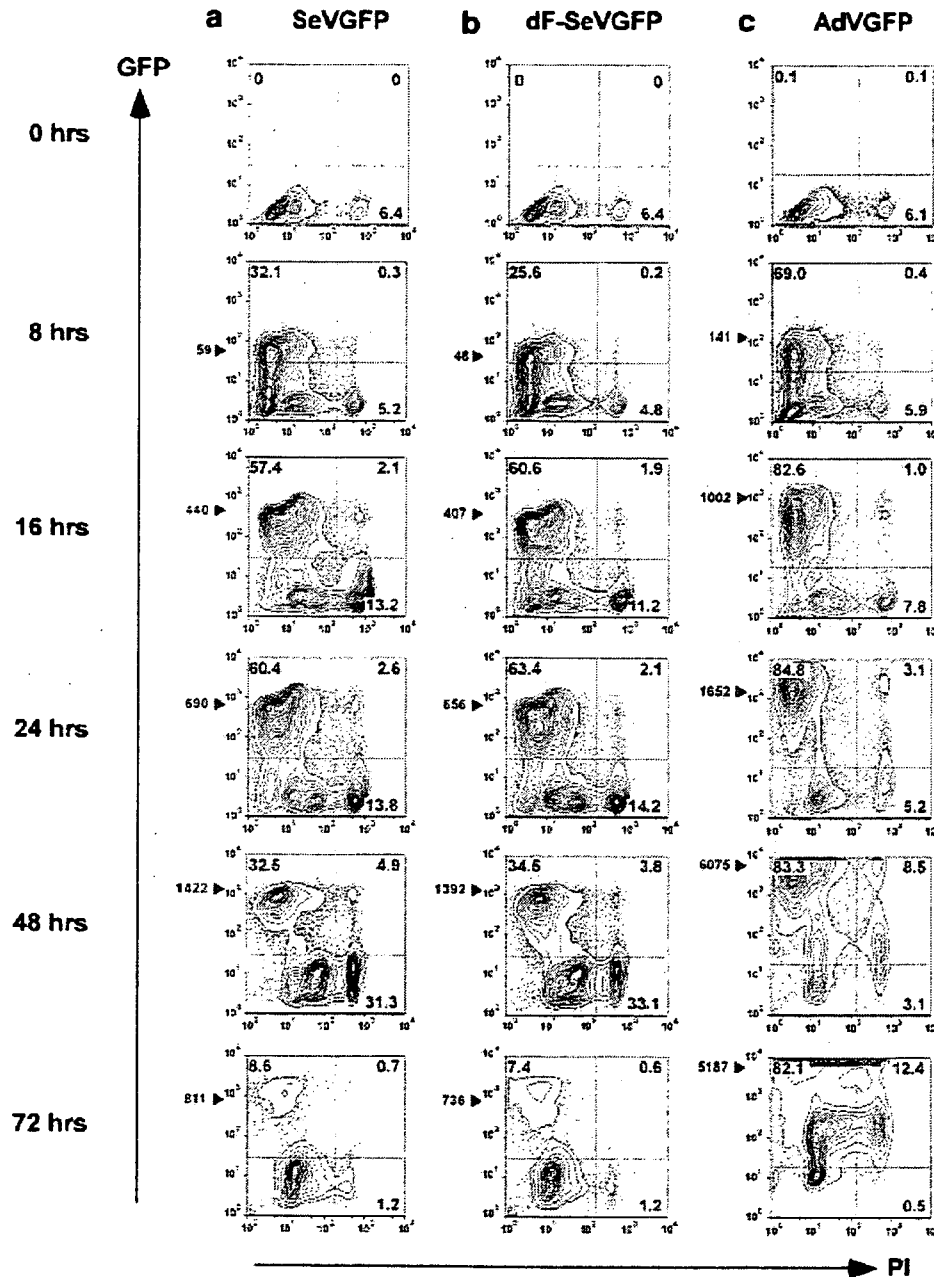


Fig. 2. The time course of the maximum gene expression by SeV (a), dF-SeV (b), and AdV (c) vectors. DCs were infected with SeV and dF-SeV at an MOI of 2 and were infected with AdV at MOI of 1,000 and then cultured for 8–72 hr. Cell viability was determined by staining with PI. GFP expression and PI staining were analyzed by flow cytometry. The percentages of GFP- and PI-positive cells are shown on each corner. Arrowheads indicate MFI of GFP-positive cells within PI-negative fraction. The numbers in each panel represent the mean value of three independent experiments.

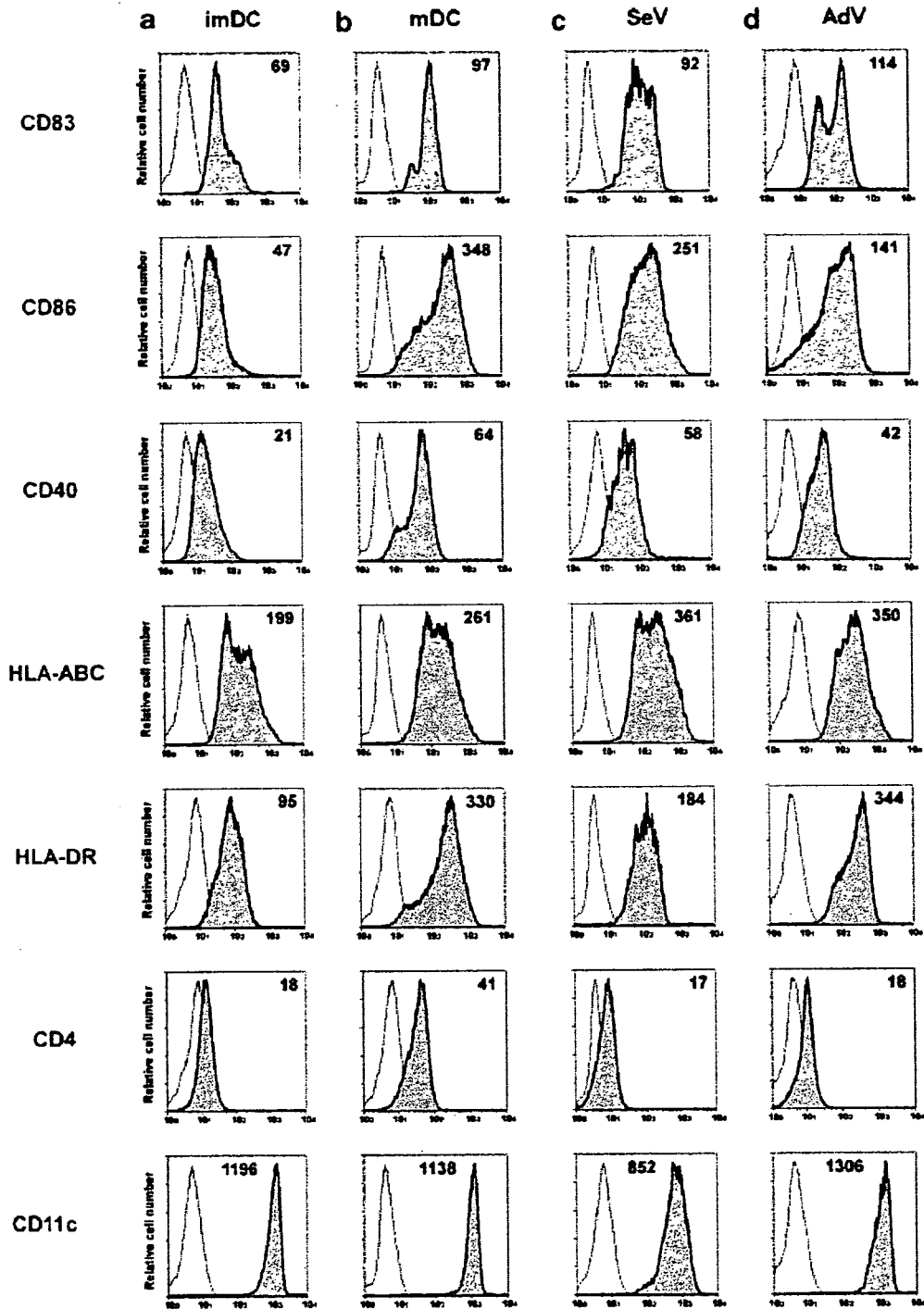


Fig. 3. Infection of DCs with SeV and AdV vectors modified the expression of cell surface markers. DCs infected with SeV vector containing no inserts at an MOI of 2 (c) and AdV vector containing no inserts at an MOI of 1,000 (d) were maintained in the DC medium for 48 hr (AdV vector) and 24 hr (SeV vector). DCs were incubated with (b) or without (a) TNF- α for 48 hr, respectively. These DCs were analyzed by flow cytometry with FITC, PE, PerCP, APC-conjugated

antibodies for expression of CD86, CD83, HLA-ABC, HLA-DR, CD4, CD40, CD11c. The open profiles represent isotype-matched mAb controls. MFIs are indicated on the right corner in each panel. The background values of all experiments were less than 15. The numbers in each panel represent the mean value of three independent experiments.

60%) 24 hr after infection with SeVGFP or dF-SeVGFP. The proportion of GFP-positive cells decreased to around 30% at 48 hr, although the MFI of GFP showed the maximum at 48 hr. AdvGFP, on the other hand, showed the maximum level of both GFP-positive cells and MFI of GFP 48 hr after infection.

Sendai and Adenoviral Vectors Changed Phenotype of imDCs Following Viral Transduction

In order to determine the effect of transduction on imDCs with these vectors, we examined the surface markers of cells after transduction. The phenotype of imDCs and mDCs are shown in Figure 3a,b, respectively. We infected imDCs with SeV or Adv vectors and cultured them for 24 or 48 hr, respectively (Fig. 3c,d). As compared with the phenotype of uninfected imDCs, DCs infected by SeV and Adv vectors showed up-regulation of a maturation marker CD83, the major histocompatibility complex (MHC) classes I and II molecules (HLA-ABC and HLA-DR), and costimulatory molecules CD40 and CD86. Incubation of DCs in medium and buffers used to prepare vectors did not affect the phenotype of the cells (data not shown). These results indicate that SeV as well as Adv vector infection induced DC maturation in terms of cell surface phenotype.

Both SeV and Adv Vectors Elicited HIV-1 Specific T-Cell Responses

To evaluate protein expressions, we developed five viral vectors carrying HIV-1 structural proteins (Fig. 4a). We infected DCs with these vectors under the optimal conditions we concluded from the results shown above. Gp120 expression by SeV vector was 3.8 times higher than that by Adv vector (Fig. 4b, compare lanes 3–7). Since 3.2 and 5.0 kb are the maximum gene sizes for SeV and Adv vector, respectively [Sakai et al., 1999; the manufacturer’s protocol of Adv Expression Kit], we inserted HIV-1 *gag* gene (about 1.5 kb) in SeV and *gag-pol* gene (about 4.9 kb) in Adv vector. Both *cis*-acting RRE sequence and *trans*-acting Rev protein were necessary for Gag protein expression by Adv vector (Fig. 4b, compare lanes 1–2). Rev expression is not required for SeV-mediated Gag or Env expression

because SeV replicates in the cytoplasm. In the presence of Rev protein, Adv vector expressed similar levels of Gag protein to SeV vector (Fig. 4b, compare lanes 1–6). Although SeV Gag did not have HIV-1 protease sequence, a band was detected near the size of p24. It was not a nonspecific band derived from SeV because we could not detect the band with other SeV constructs, such as SeV Env (data not shown). Gag might be processed by some proteins of SeV.

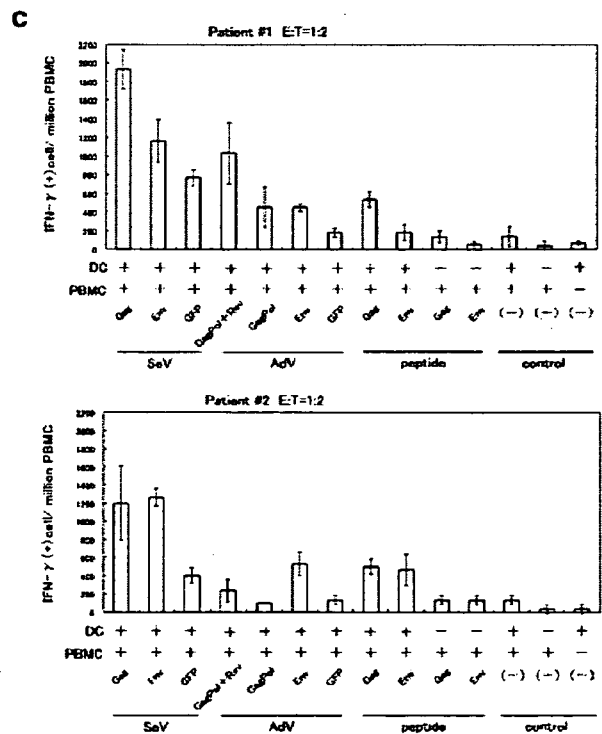
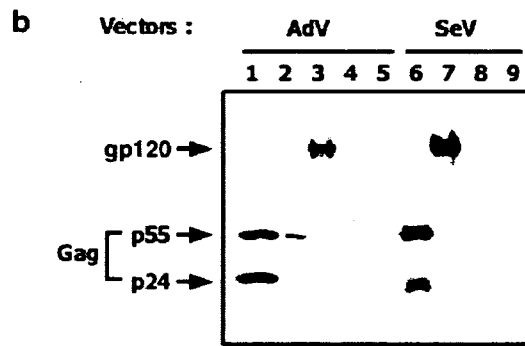
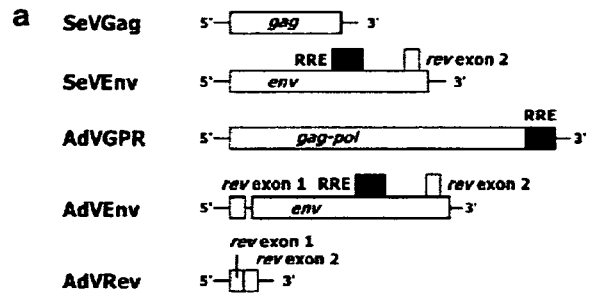


Fig. 4. a: Schematic structures of five viral vectors. *gag*, HIV-1 gag gene; *env*, HIV-1 env gene; *gag-pol*, HIV-1 gag and pol genes; *rev*, HIV-1 rev gene; RRE, HIV-1 RRE; GFP, green fluorescent protein. b: The expression of HIV-1 structural proteins by Adv and SeV vectors. DCs infected with Adv vector at an MOI of 1,000 and SeV vector at an MOI of 2 were harvested for 48 or 24 hr after infection, respectively. Thirty microgram of lysate was subjected to immunoblot analysis using anti-Gag p24 or anti-Env gp120 mAbs. The other two independent experiments showed similar results. Lane 1, coinfection with AdvGPR and AdvRev; Lane 2, AdvGPR without AdvRev infection; Lane 3, AdvEnv; Lane 4, Adv without inserts; Lane 5, mock; Lane 6, SeVGag; Lane 7, SeVEnv; Lane 8, SeV without inserts; Lane 9, mock. Arrows on the left indicate positions of gp120, Gag p55, and p24. c: The results of IFN- γ Elispot assays in two HIV-1 infected patients. Autologous DCs infected with SeV vector or Adv vector, or just added overlapping peptides (*gag*, *env*) were used as stimulators. PBMCs from the same patients were used as effector cells. Results are shown as mean \pm SEM of three independent assays.

After developing mDCs from frozen PBMCs of two HIV-1 infected patients, we infected these mDCs with SeV or AdV vector and used them as stimulators for interferon γ (IFN- γ) Elispot. Both SeV and AdV vectors elicited HIV-1 specific T-cell responses, although some nonspecific responses were also detected (Fig. 4c).

DISCUSSION

DCs are efficient antigen presenting cells that are critical for induction of primary T-cell responses. At present the most useful method for genetic manipulation of DCs is to use viral vectors. As reported previously, AdV vector is efficient at the transduction of DCs [Tan et al., 2005]. SeV is also one of the reliable vectors for immunotherapy and has several unique features, such as cytoplasmic localized replication cycle and brief contact time for cellular uptake. In this study, we analyzed the capacity of SeV as a vector in terms of transducing GFP and HIV-1 genes into human DCs. We showed that SeV vector transduced GFP genes efficiently into monocyte-derived imDCs. DCs infected with SeV and dF-SeV vectors expressed high amount of GFP gene 24 hr after infection at an MOI of 2 (Fig. 2a,b). The expression level of HIV-1 structural gene, *env*, by SeV vector was higher than that by AdV. These results proved the high ability of gene expression by SeV. However, the proportion of GFP positive cells did not increase according to MOI. About 30% of cells were still GFP-negative 48 hr after infection even at an MOI of 50 (Fig. 1a,b). This could be caused by the disruption of sialic acid which is the receptor for SeV.

Both SeV and dF-SeV vectors killed nearly 30% of target DCs at the lowest MOI: 0.5. One of the reasons for this phenomenon is likely to be apoptosis. Several studies reported that SeV is able to induce apoptosis in viral host cells [Tropea et al., 1995; Bitzer et al., 1999]. This cytopathic effect might enhance specific T-cell responses by cross-presentation of DCs. Presentation by DCs derived from virus-infected apoptotic and necrotic cells could activate T-cells efficiently [Arrode et al., 2000; Herr et al., 2000; Larsson et al., 2001; Tabi et al., 2001]. In order to apply SeV in a clinical setting, further studies about cytopathic effect by SeV vector will be required.

AdV vector is known to require high MOI to achieve high transduction rates [Diao et al., 1999]. Our study also demonstrated that much higher MOI was needed in AdV than SeV to transduce DCs. One of the reasons for this phenomenon could be insufficient expression of coxsackievirus and AdV receptor (CAR) [Stockwin et al., 2002] on DCs. CAR is the primary receptor for AdV type 5, and the AdV used in this study was derived from AdV type 5. However, MFI of GFP in AdV vector increased according to MOI. AdV might be able to use other receptors to infect DCs. Several studies have shown that AdV can infect cells through integrins or MHC molecules [Huang et al., 1996; Hong et al., 1997]. Recently, AdV vector containing Ad5/35 chimeric fiber protein was reported as a useful vector for the cells lacking in sufficient CAR expression [Mizuguchi and Hayakawa,

2002]. This chimeric vector would be useful for DCs because the receptor of Ad5/35 vector is CD46, which is expressed on DCs.

When imDCs capture antigens, they mature while migrating to T-cell areas in the lymph nodes [Banchereau and Steinman, 1998]. DC maturation is critical for strong T-cell binding and stimulation [Lipscomb and Masten, 2002]. Our results showed that SeV vector infection induced DC maturation of human monocyte-derived DCs as well as AdV vector infection. However, the expression levels of CD86 and CD40 were lower as compared to those of mDCs. CD40 expression leads to increased DC survival and stimulates cytokine production [Caux et al., 1994; Wong et al., 1997]. CD86, a ligand for CD28 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), stimulates T-cell proliferation and generation of CTL [Lanier et al., 1995]. In order to achieve further up-regulation of those molecules, the addition of a maturation factor, such as TNF- α , should be considered.

DCs infected with SeV as well as AdV elicited HIV-1 specific T-cell responses detected by IFN- γ Elispot (Fig. 4c). Elispot by SeV GFP showed about 800 SFC/million PBMC which was obtained from patient #1. One possibility of this nonspecific response is antigenic cross-reactivity. SeV belongs to the genus *Respirovirus* of the *Paramyxoviridae* family. *Respirovirus* includes human parainfluenza virus type 1 (hPIV-1) and 3 (hPIV-3). hPIV-1 is the most common cause of pediatric laryngotracheobronchitis (croup), which means many people are infected by hPIV-1 in early life. Previous studies showed SeV and hPIV-1 shared sequence homology and antigenic cross-reactivity [Gorman et al., 1990; Lyn et al., 1991; Smith et al., 1994]. The high nonspecific response by SeV GFP could be caused by cross-reactive immunity induced by previous exposure to human hPIV-1.

In conclusion, our results showed that SeV vector had high ability of gene transduction. SeV vector induced the maturation of DCs in terms of their phenotype and stimulated HIV-1 specific T-cell responses, which is beneficial in vaccination. Though further studies will be required to improve vector design, SeV vector has a potential to be used for immuno-genotherapy.

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REFERENCES

- Agungpriyono DR, Yamaguchi R, Uchida K, Tohya Y, Kato A, Nagai Y, Asakawa M, Tateyama S. 2000. Green fluorescent protein gene insertion of Sendai Virus infection in nude mice: Possibility as an infection tracer. *J Vet Med Sci* 62:223–228.
- Arrode G, Boccaccio C, Lule J, Allart S, Moinard N, Abastado JP, Alam A, Davrinche C. 2000. Incoming human cytomegalovirus pp65 (UL83) contained in apoptotic infected fibroblasts is cross-presented to CD8(+) T cells by dendritic cells. *J Virol* 74:10018–10024.
- Banchereau J, Steinman RM. 1998. Dendritic cells and the control of immunity. *Nature* 392:245–252.
- Bitzer M, Prinz F, Bauer M, Spiegel M, Neubert WJ, Gregor M, Schulze-Osthoff K, Lauer U. 1999. Sendai virus infection induces apoptosis through activation of caspase-8 (FLICE) and caspase-3 (CPP32). *J Virol* 73:702–708.
- Bonini C, Lee SP, Riddell SR, Greenberg PD. 2001. Targeting antigen in mature dendritic cells for simultaneous stimulation of CD4+ and CD8+ T cells. *J Immunol* 166:5250–5257.
- Brander C, Walker BD. 1999. T lymphocyte responses in HIV-1 infection: Implications for vaccine development. *Curr Opin Immunol* 11:451–459.
- Caux C, Massacrier C, Vanbervliet B, Dubois B, Van Kooten C, Durand I, Banchereau J. 1994. Activation of human dendritic cells through CD40 cross-linking. *J Exp Med* 180:1263–1272.
- Diao J, Smythe JA, Smyth C, Rowe PB, Alexander IE. 1999. Human PBMc-derived dendritic cells transduced with an adenovirus vector induce cytotoxic T-lymphocyte responses against a vector-encoded antigen in vitro. *Gene Ther* 6:845–853.
- Engelmayer J, Larsson M, Subklewe M, Chahroudi A, Cox WI, Steinman RM, Bhardwaj N. 1999. Vaccinia virus inhibits the maturation of human dendritic cells: A novel mechanism of immune evasion. *J Immunol* 163:6762–6768.
- Engelmayer J, Larsson M, Lee A, Lee M, Cox WI, Steinman RM, Bhardwaj N. 2001. Mature dendritic cells infected with canarypox virus elicit strong anti-human immunodeficiency virus CD8+ and CD4+ T-cell responses from chronically infected individuals. *J Virol* 75:2142–2153.
- Furutsuki T, Hosoya N, Kawana-Tachikawa A, Tomizawa M, Odawara T, Goto M, Kitamura Y, Nakamura T, Kelleher AD, Cooper DA, Iwamoto A. 2004. Frequent transmission of cytotoxic-T-lymphocyte escape mutants of human immunodeficiency virus type 1 in the highly HLA-A24-positive Japanese population. *J Virol* 78:8437–8445.
- Gorman WL, Gill DS, Scroggs RA, Portner A. 1990. The hemagglutinin-neuraminidase glycoproteins of human parainfluenza virus type 1 and Sendai virus have high structure-function similarity with limited antigenic cross-reactivity. *Virology* 175:211–221.
- Graham FL, Smiley J, Russell WC, Nairn R. 1977. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J Gen Virol* 36:59–74.
- Herr W, Ranieri E, Olson W, Zarour H, Gesualdo L, Storkus WJ. 2000. Mature dendritic cells pulsed with freeze-thaw cell lysates define an effective in vitro vaccine designed to elicit EBV-specific CD4(+) and CD8(+) T lymphocyte responses. *Blood* 96:1857–1864.
- Hong SS, Karayan L, Tournier J, Curriel DT, Boulanger PA. 1997. Adenovirus type 5 fiber knob binds to MHC class I alpha2 domain at the surface of human epithelial and B lymphoblastoid cells. *EMBO J* 16:2294–2306.
- Huang S, Kamata T, Takada Y, Ruggeri ZM, Nemerow GR. 1996. Adenovirus interaction with distinct integrins mediates separate events in cell entry and gene delivery to hematopoietic cells. *J Virol* 70:4502–4508.
- Ishii-Watabe A, Uchida E, Iwata A, Nagata R, Satoh K, Fan K, Murata M, Mizuguchi H, Kawasaki N, Kawanishi T, Yamaguchi T, Hayakawa T. 2003. Detection of replication-competent adenoviruses spiked into recombinant adenovirus vector products by infectivity PCR. *Mol Ther* 8:1009–1016.
- Jin X, Bauer DE, Tuttleton SE, Lewin S, Gettie A, Blanchard J, Irwin CE, Safrit JT, Mittler J, Weinberger L, Kostrikis LG, Zhang L, Perelson AS, Ho DD. 1999. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. *J Exp Med* 189:991–998.
- Kanegae Y, Makimura M, Saito I. 1994. A simple and efficient method for purification of infectious recombinant adenovirus. *Jpn J Med Sci Biol* 47:157–166.
- Kano M, Matano T, Kato A, Nakamura H, Takeda A, Suzuki Y, Ami Y, Terao K, Nagai Y. 2002. Primary replication of a recombinant Sendai virus vector in macaques. *J Gen Virol* 83:1377–1386.
- Kato A, Sakai Y, Shioda T, Kondo T, Nakanishi M, Nagai Y. 1996. Initiation of Sendai virus multiplication from transfected cDNA or RNA with negative or positive sense. *Genes Cells* 1:569–579.
- Kato M, Igarashi H, Takeda A, Sasaki Y, Nakamura H, Kano M, Sata T, Iida A, Hasegawa M, Horie S, Higashihara E, Nagai Y, Matano T. 2005. Induction of Gag-specific T-cell responses by therapeutic immunization with a Gag-expressing Sendai virus vector in macaques chronically infected with simian-human immunodeficiency virus. *Vaccine* 23:3166–3173.
- Kawana-Tachikawa A, Tomizawa M, Nunoya J, Shioda T, Kato A, Nakayama EE, Nakamura T, Nagai Y, Iwamoto A. 2002. An efficient and versatile mammalian viral vector system for major histocompatibility complex class I/peptide complexes. *J Virol* 76:11982–11988.
- Lanier LL, O'Fallon S, Somoza C, Phillips JH, Linsley PS, Okumura K, Ito D, Azuma M. 1995. CD80 (B7) and CD86 (B70) provide similar costimulatory signals for T cell proliferation, cytokine production, and generation of CTL. *J Immunol* 154:97–105.
- Larsson M, Fonteneau JF, Somersan S, Sanders C, Bickham K, Thomas EK, Mahnke K, Bhardwaj N. 2001. Efficiency of cross presentation of vaccinia virus-derived antigens by human dendritic cells. *Eur J Immunol* 31:3432–3442.
- Levy JA, Cheng-Mayer C, Dina D, Luciw PA. 1986. AIDS retrovirus (ARV-2) clone replicates in transfected human and animal fibroblasts. *Science* 232:998–1001.
- Li HO, Zhu YF, Asakawa M, Kuma H, Hirata T, Ueda Y, Lee YS, Fukumura M, Iida A, Kato A, Nagai Y, Hasegawa M. 2000. A cytoplasmic RNA vector derived from nontransmissible Sendai virus with efficient gene transfer and expression. *J Virol* 74:6564–6569.
- Lipscomb MF, Masten BJ. 2002. Dendritic cells: Immune regulators in health and disease. *Physiol Rev* 82:97–130.
- Lyn D, Gill DS, Scroggs RA, Portner A. 1991. The nucleoproteins of human parainfluenza virus type 1 and Sendai virus share amino acid sequences and antigenic and structural determinants. *J Gen Virol* 72:983–987.
- Matano T, Shibata R, Siemon C, Connors M, Lane HC, Martin MA. 1998. Administration of an anti-CD8 monoclonal antibody interferes with the clearance of chimeric simian/human immunodeficiency virus during primary infections of rhesus macaques. *J Virol* 72:164–169.
- McMichael AJ, Rowland-Jones SL. 2001. Cellular immune responses to HIV. *Nature* 410:980–987.
- Miyake S, Makimura M, Kanegae Y, Harada S, Sato Y, Takamori K, Tokuda C, Saito I. 1996. Efficient generation of recombinant adenoviruses using adenovirus DNA-terminal protein complex and a cosmid bearing the full-length virus genome. *Proc Natl Acad Sci USA* 93:1320–1324.
- Mizuguchi H, Hayakawa T. 2002. Adenovirus vectors containing chimeric type 5 and type 35 fiber proteins exhibit altered and expanded tropism and increase the size limit of foreign genes. *Gene* 285:69–77.
- Mwau M, Cebere I, Sutton J, Chikoti P, Winstone N, Wee EG, Beattie T, Chen YH, Dorrell L, McShane H, Schmidt C, Brooks M, Patel S, Roberts J, Conlon C, Rowland-Jones SL, Bwayo JJ, McMichael AJ, Hanke T. 2004. A human immunodeficiency virus 1 (HIV-1) clade A vaccine in clinical trials: Stimulation of HIV-specific T-cell responses by DNA and recombinant modified vaccinia virus Ankara (MVA) vaccines in humans. *J Gen Virol* 85:911–919.
- Nagayama H, Sato K, Morishita M, Uchimaruk K, Oyaizu N, Inazawa T, Yamasaki T, Enomoto M, Nakaoka T, Nakamura T, Maekawa T, Yamamoto A, Shimada S, Saida T, Kawakami Y, Asano S, Tani K, Takahashi TA, Yamashita N. 2003. Results of a phase I clinical study using autologous tumour lysate-pulsed monocyte-derived mature dendritic cell vaccinations for stage IV malignant mela-

- noma patients combined with low dose interleukin-2. *Melanoma Res* 13:521–530.
- Rea D, Schagen FH, Hoeben RC, Mehtali M, Havenga MJ, Toes RE, Melief CJ, Offringa R. 1999. Adenoviruses activate human dendritic cells without polarization toward a T-helper type 1-inducing subset. *J Virol* 73:10245–10253.
- Rouas R, Uch R, Cleuter Y, Jordier F, Bagnis C, Mannoni P, Lewalle P, Martiat P, Van den Broeke A. 2002. Lentiviral-mediated gene delivery in human monocyte-derived dendritic cells: Optimized design and procedures for highly efficient transduction compatible with clinical constraints. *Cancer Gene Ther* 9:715–724.
- Sakai Y, Kiyotani K, Fukumura M, Asakawa M, Kato A, Shioda T, Yoshida T, Tanaka A, Hasegawa M, Nagai Y. 1999. Accommodation of foreign genes into the Sendai virus genome: Sizes of inserted genes and viral replication. *FEBS Lett* 456:221–226.
- Smith FS, Portner A, Leggiadro RJ, Turner EV, Hurwitz JL. 1994. Age-related development of human memory T-helper and B-cell responses toward parainfluenza virus type-1. *Virology* 205:453–461.
- Stockwin LH, Matzow T, Georgopoulos NT, Stanbridge LJ, Jones SV, Martin IG, Blair-Zajdel ME, Blair GE. 2002. Engineered expression of the Coxsackie B and adenovirus receptor (CAR) in human dendritic cells enhances recombinant adenovirus-mediated gene transfer. *J Immunol Methods* 259:205–215.
- Stubbs AC, Martin KS, Coeshott C, Skaates SV, Kuritzkes DR, Bellgrau D, Franzusoff A, Duke RC, Wilson CC. 2001. Whole recombinant yeast vaccine activates dendritic cells and elicits protective cell-mediated immunity. *Nat Med* 7:625–629.
- Tabi Z, Moutafsi M, Borysiewicz LK. 2001. Human cytomegalovirus pp65- and immediate early 1 antigen-specific HLA class I-restricted cytotoxic T cell responses induced by cross-presentation of viral antigens. *J Immunol* 166:5695–5703.
- Takeda A, Igarashi H, Nakamura H, Kano M, Iida A, Hirata T, Hasegawa M, Nagai Y, Matano T. 2003. Protective efficacy of an AIDS vaccine, a single DNA priming followed by a single booster with a recombinant replication-defective Sendai virus vector, in a macaque AIDS model. *J Virol* 77:9710–9715.
- Tan PH, Beutelspacher SC, Xue SA, Wang YH, Mitchell P, McAlister JC, Larkin DF, McClure MO, Stauss HJ, Ritter MA, Lombardi G, George AJ. 2005. Modulation of human dendritic-cell function following transduction with viral vectors: Implications for gene therapy. *Blood* 105:3824–3832.
- Tropea F, Troiano L, Monti D, Lovato E, Malorni W, Rainaldi G, Mattana P, Viscomi G, Ingletti MC, Portolani M, et al. 1995. Sendai virus and herpes virus type 1 induce apoptosis in human peripheral blood mononuclear cells. *Exp Cell Res* 218:63–70.
- Tsunetsugu-Yokota Y, Morikawa Y, Isogai M, Kawana-Tachikawa A, Odawara T, Nakamura T, Grassi F, Autran B, Iwamoto A. 2003. Yeast-derived human immunodeficiency virus type 1 p55(gag) virus-like particles activate dendritic cells (DCs) and induce perforin expression in Gag-specific CD8(+) T cells by cross-presentation of DCs. *J Virol* 77:10250–10259.
- Wong BR, Josien R, Lee SY, Sauter B, Li HL, Steinman RM, Choi Y. 1997. TRANCE (tumor necrosis factor [TNF]-related activation-induced cytokine), a new TNF family member predominantly expressed in T cells, is a dendritic cell-specific survival factor. *J Exp Med* 186:2075–2080.
- Yang OO, Kalams SA, Trocha A, Cao H, Luster A, Johnson RP, Walker BD. 1997. Suppression of human immunodeficiency virus type 1 replication by CD8+ cells: Evidence for HLA class I-restricted triggering of cytolytic and noncytolytic mechanisms. *J Virol* 71:3120–3128.
- Yu D, Shioda T, Kato A, Hasan MK, Sakai Y, Nagai Y. 1997. Sendai virus-based expression of HIV-1 gp120: Reinforcement by the V(-) version. *Genes Cells* 2:457–466.

厚生労働科学エイズ対策研究事業

「血友病の治療とその合併症の克服に関する研究（主任研究者：坂田洋一）」分担研究

「血液凝固異常症のQOLに関する研究」

平成19年度調査報告書

「血液凝固異常症のQOLに関する研究 平成19年度調査報告書」の刊行に際して

この度、平成19年度厚生労働科学エイズ対策研究事業「血友病の治療とその合併症の克服に関する研究（主任研究者：坂田洋一）」の分担研究として行われた「血液凝固異常症のQOLに関する研究 平成19年度調査報告書」の報告書が出来上がりましたのでお送り申し上げます。

本調査の実施に際しては、御多忙中にも拘わらずQOL調査票に御記入頂き、返送して頂いた患者さんおよびその御家族の方に深謝申し上げます。また、本調査票の配布を仲介して頂いた患者組織および担当医の皆様にも厚く御礼申し上げます。本調査は、患者さんおよび御家族の方々の治療および生活の質の向上に寄与できることを目的に行われました。今回は一次解析結果を御報告致しますが、今後更に本調査結果を解析し、さらに有用な情報提供、そして提言を行ってまいり所存でございますので、よろしく御協力の程御願い申し上げます。今回の調査で、定年後（老後）の問題点に関する調査項目が欠落していると指摘されました。今後、このような調査の機会がありましたら調査項目に加え検討させていただきます。

平成20年2月吉日

平成19年度厚生労働科学エイズ対策研究事業「血友病の治療とその合併症の克服に関する研究（主任研究者：坂田洋一）」分担研究：「血液凝固異常症のQOLに関する研究」

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I. 要望・提言

1. 自由記載欄に寄せられた行政・医療従事者・製薬企業への要望

1) 血友病治療について

血友病治療に対する患者・家族の究極の願いは、遺伝子治療などによる病気の治癒である。中には、遺伝子治療による患者自身の治療だけでなく、血友病の遺伝子が子どもへ伝わることなくなる治療を期待するコメントもあった。しかし、遺伝子治療にはこれから解決しないといけない様々な問題が内蔵することから、自由記載欄には現実的な観点に立ち、安全で利便性の高い製剤を安定供給して欲しいという意見が多数寄せられた。とくに多かった要望は利便性の高い製剤の開発で、(1)静脈注射をしなくても良い製剤として経口薬や坐薬の開発、(2)静脈注射が必要な場合は作用時間(半減期)の長い製剤の開発、(3)常温で保存できる製剤の開発などである。安全性については、ヒト免疫不全ウイルス、C型肝炎ウイルスなど血液で媒介されうる感染症に対する万全の対策を望むとともに、いまだ我が国では使うことのできない遺伝子組換え第Ⅸ因子製剤の早期認可を要望する声があった。遺伝子組換え第Ⅷ因子製剤についても安全供給の観点から我が国での製造を期待する意見があった。

製剤の止血効果や副作用についての具体的なコメントはなかったが、例外はインヒビター患者に対する止血製剤の効果で、インヒビターを保有しない血液患者の補充療法と比べて止血効果が大幅に劣ることが指摘された。また、製剤が高価なため、必要な手術を受けられないという深刻な問題も提起された。

その他、凝固因子製剤により感染したC型慢性肝炎に苦しんでいる多くの患者から、さらに強力な治療薬の開発を望むとの意見が寄せられた。

これらの問題に対しては、製薬企業のみならず行政と専門医の三者が協力して取り組む必要がある。

2) 血友病医療体制について

日本全国でも5000人と患者が少ないことから、血友病のことをよく知らない医師の診察を受けた患者・家族からかなりの苦情が寄せられ、血友病のわかる医師を増やす必要が指摘された。中には、血友病専門医の高齢化を心配し、若い血友病専門医を増やして欲しいという意見もあった。医療者については、血友病専門医(内科医、小児科医)のみならず、血友病に詳しい整形外科医、歯科・口腔外科医、看護師を養成して欲しいという要望が寄せられた。さらに、カウンセラーやソーシャルワーカーも配置した血友病センターを各地に設置して、医療者間の連携システムを構築することが提案された。また、病院の統廃合が進む中、地域による医療格差が益々拡大していることへの危惧を示すコメントもあった。

3) 医療保障について

行政に対する最も強い要望は、現在の医療費公費負担制度の継続である。とくに今回の調査では老後の不安を訴える意見が多く、老後のサポート体制を含めて、医療費負担やその他のサポート体制について、政府から展望の持てるプランを示す必要がある。

更新手続きの簡素化についても多くの意見があった。遺伝性疾患であり、治癒の見込みがない疾患であるにもかかわらず、毎年、毎年継続申請をしなければならず、診断書代金がかかり、仕事も休まなければならないことに対する苦情、不満が多数寄せられた。毎年、診断書を書くことには医師の側でも大きな負担になっており、行政側は簡素化を検討する必要がある。また、手続きに際して、プライバシーの保護に対する配慮が不十分なことなど、役所の窓口への対応に不満が寄せられた。

4) 社会生活上の問題

行政、医療従事者、製薬企業に対して直接寄せられたものではないが、生命保険への加入、就学、就職、結婚など社会生活上の様々な問題は、病気への無理解によるものが少なからずあるので、国民の血友病への理解を深める方策を講じて欲しいという意見があった。