doubt improve the kinetics of Ad vectors to better meet the requirements of clinical trial.

4.3. Cationic liposome complexation or PEGylation

The most straightforward approach for circumventing Ad-induced immunogenic toxicities has been the use of cationic liposome and polyethylene glycol (PEG) to shield the vectors from the host immune system.

Cationic liposomes have shown promise as a gene delivery tool for plasmid DNA due to their simplicity, safety and efficiency in some tissues [131]. Several groups have observed that cationic liposome-conjugated Ad vectors greatly increase gene transduction efficiency to a variety of cell and tissue types that are especially resistant to Ad infection, including vascular tissue [132], human smooth muscle cells [133], airway epithelia [134] and human glioma cells [135]. Fasbender et al. [134] suggested that viral binding was dependent on an electrostatic interaction with the cell surface, that viral entry did not require an interaction of the Ad fiber protein with the cell surface, and that cationic liposome-conjugated Ad vectors entered cells via a pathway different from that utilized by Ad alone. Use of a more efficient delivery system could allow a smaller dose of Ad vector to be administered for therapeutic effects, thereby decreasing the total immune response. Yoshida's group noted that mice injected with cationic liposome-conjugated Ad vectors produced fewer anti-Ad antibodies compared with an equivalent dose of unconjugated Ad vectors, and cationic liposome-conjugated Ad vectors were less susceptible to inactivation by neutralizing antibodies than Ad vectors alone [136,137]. The increased transduction efficiency, reduced antigenicity, and attenuated susceptibility to neutralizing antibodies might be beneficially multiplied for redosing.

Complexation with PEG (PEGylation) is frequently used in pharmaceutic preparations to provide a hydrophilic coat and to increase blood persistence of therapeutic proteins such as erythropoietin (EPO), granulocyte macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor-α (TNF-α) etc. [138–140]. Covalent attachment of PEG to the surface of the Ad vector might prolong persistence in the blood and circumvent neutralization of Ad vectors by antibodies [141]. There is a report that PEGylated Ad vectors exhibited a 4-fold slower clearance rate than the non-PEGylated Ad vectors [38]. Furthermore, coupling of PEG to the viral capsids attenuates the ability of the vector to infect antigen presenting cells, thereby reducing inflammatory responses. Animals administered with the PEGylated Ad vectors exhibited reduced levels of both cell-mediated and humoral immune responses, resulting in significant gene expression upon readministration of unmodified Ad vectors in the lung [141,142]. However, PEGylation might lead to loss of infectivity [38]. The activated PEG reacts preferentially with the ε-amino terminal of lysine residues on the capsid, including the hexon, fiber and penton base, which might lead to decreased infectivity. Experimental optimization of the PEGylation reaction so that PEG occupies only 70 to 80% of the available sites on virus capsid proteins shielded vectors from antibody neutralization and retained viral infectivity [143,144]. Nonetheless, compared with local readministration, the results of systemic readministration of the PEGylated Ad vectors were less encouraging. Levels of transgene expression achieved by systemic readministration of the PEGylated Ad vectors were on the order of 1000- to 10,000-fold lower than that seen in animals carrying no neutralizing antibodies against Ad; these levels were also significantly lower than those observed with readministration of these modified vectors in the lung [142]. Thus, the exact nature of the immune response against the PEG modified Ad must be characterized further.

4.4. Immune intervention

The success of long-term gene therapy by Ad vectors depends on finding ways to avoid/attenuate the induction of immune responses to both the vector and the transgene product. Some advances have been made in the development of novel strategies to disrupt or modulate immune responses in various animal models; these approaches include inhibiting cell-mediated immune responses to prolong transgene expression, and inhibiting humoral immune responses to permit readministration of the vector.

One straightforward approach is to disrupt the inflammatory immune response by inhibition of NF κB activation, thereby blocking release of inflammatory cytokines like TNF-α and IL-6 [145,146]; other relatively simple approaches include pretreatment by

or coadministration of anti-inflammatory drugs such as steroids [147–150]. Lieber et al. [145] obtained persistent Ad-delivered hAAT [human alpha (1)-antitrypsin] expression in bcl-2 transgenic mice for longer than 3 months by coadministrating an Ad vector expressing an IκBα supersuppressor to unravel the role of virus-induced NF κB activation [151,152]. They found that to confer vector persistence, simultaneous expression of bcl-2, an antiapoptotic protein [153], was required to block virus-induced apoptosis, while NF κB protection was inactivated by the IκBα supersuppressor. Kolb et al. [147] investigated the use of topical corticosteroids in improving gene expression after repeated injection of Ad vectors into mouse lungs. They showed that budesonide given around exposure to Ad to the lung significantly helped maintain high levels of the expressed transgene protein in bronchoalveolar lavage fluid after as many as four consecutive injections of virus at 2-week intervals. Furthermore, they observed that the improved transgene expression in budesonide-treated animals was associated with a reduction, but not prevention of neutralizing antiviral antibodies.

Another approach is the use of immunosuppressive drugs to inhibit cell-mediated immune functions, just as they are used in clinical trials for organ transplantation. The central role of CD4+ T cells in the activation of cellular and humoral immune response has focused immunosuppressive strategies towards blockade of costimulatory molecules. Recombinant murine CTLA4Ig (an inhibitor of the CD28/B7 pathway) and the anti-CD40 ligand antibody block costimulatory interactions between T cells and antigen presenting cells. Blocking of costimulatory signals has been shown to inhibit T-cell activation in several animal models of Ad vector gene therapy. Kay et al. [154] observed that when the anti-CD40 ligand and recombinant murine CTLA4Ig were coadministered around the time of primary Ad vector administration, Ad-mediated gene expression was maintained up to 1 year in mouse livers, and persistent secondary Ad-mediated gene expression lasted for at least 200-300 days even after the immunosuppressive effects of these agents were no longer present. However, neither agent alone allowed transduction after secondary vector administration. Jooss et al. [155] described a strategy that aimed to inhibit CD4+ T cell activation by transiently administering CTLA4Ig at the time an E1-deleted Ad vector is administered to the liver or lung. In the lung, CTLA4Ig treatment significantly blocked the formation of neutralizing antibodies, allowing efficient readministration of the vector, whereas transgene expression was only moderately prolonged. In contrast, CTLA4Ig did not suppress neutralizing antibody formation in the context of liver gene therapy, but resulted in more stable gene expression. These observations suggest that it may be possible to obtain persistence as well as secondary Ad-mediated gene transfer with transient inhibition of the CD28/B7 pathway at the time of virus instillation, especially in a local route. However, immune intervention in systemic Ad administration seems to be much complex. Moreover, this immunomodulation might compromise the host immune response. Thus, the clinical utility is uncertain.

4.5. Optimization of in-cis acting elements

As discussed in the previous section, the immunogenic toxicities of Ad vectors can lead to clearance of the transduced cells and only transient transgene expression. The severity of the immunogenic toxicities is intimately connected with vector dose. However, to achieve anatomically detectable and physiologically relevant levels of transgene expression, the number of Ad particles to be injected is always too large to avoid severe immunological side effects [156,157]. One simple but practical way to alleviate this kind of immunogenic toxicities is to improve the expression efficiency of each viral particle unit so that the number of Ad particles to be injected can be decreased.

Transgene expression can be modulated at both the transcriptional and post-transcriptional levels. We systematically investigated the ability of transcriptional regulatory elements [promoter, enhancer, intron and poly(A) sequences] and post-transcriptional regulatory elements to maximize the transgene expression efficiency from each Ad vector unit [158]. Our optimized Ad vector, Ad-WCMVL2, with an expression cassette containing the human CMV promoter/enhancer, intron A, the luciferase gene, the Woodchuck hepatitis virus post-transcriptional regulation element (WPRE), and bovine growth hormone (BGH) poly (A), showed more than 700-fold luciferase expression in mouse liver than the Ad vector, Ad-

CMVL1, with an expression cassette containing only the human CMV promoter/enhancer, the luciferase gene and BGH poly (A), when they were intravenously administered into the mouse [158]. It may be deduced that to express transgenes at a therapeutic level, the in-cis acting elements of our optimized Ad vector, Ad-WCMVL2, may decrease, by several orders of magnitude, the number of viral particles that must be injected as compared with Ad-CMVL1 with the expression cassette containing only the CMV promoter/enhancer and the BGH poly(A) as in-cis acting elements which are considered to be one of the most strongest in-cis acting element combination and are widely used in clinical trials and gene function studies [159]. It also means that immunogenic toxicities induced by Ad particles could be greatly alleviated by using the in-cis acting elements of Ad-WCMVL2 to construct therapeutic Ad vectors. Gerdes et al. [160] demonstrated that the murine CMV promoter was much stronger than the human CMV promoter in the context of Ad vectors in all the cell lines tested, including nonhuman primate and human cell lines. By using this murine CMV promoter, the investigators observed that the Ad vector dose required to achieve sufficient transduction could be reduced 100-fold and the cellular inflammation and viral cytotoxicity associated with the delivery of Ad vectors into the rat brain could be completely eliminated.

In the context of HD Ad vectors, the inclusion of a human centromeric region and a matrix attachment region as in-cis acting elements improved the maintenance of the Ad vector genome and the transgene expression level [57]. It can be concluded that even for the Ad vectors that have an extremely high transduction efficiency, the in-cis acting elements can be optimized to obtain maximized transgene expression.

4.6. Integrating Ad vectors

For replication-deficient Ad vectors, the existence of episomal Ad DNA presents the disadvantage of not integrating for long-term expression of the delivered foreign gene. In the case that stable genetic alternation needs to be maintained in dividing cells, integrating vectors are at present the tools of choice. Though integration is not a guarantee of stable transcription, since transgene expression from integrated vector genomes can be gradually silenced over time in some cases [161], at present the best way to get long-term transgene expression is to integrate the foreign gene into the host genome.

To incorporate integration machinery to Ad, a variety of hybrid vectors combining the highly efficient DNA delivery of Ad with the integrating machinery of adeno-associated viruses (AAV), retroviruses, and transposons have been emerging [162]. The unique biology of AAV has stimulated considerable efforts toward the development of AAV-based integrating Ad vectors. Such integrating Ad-AAV hybrid vectors devoid of all Ad viral genes were successfully developed by Lieber et al. [163] and Recchia et al. [164]. Lieber's hybrid Ad-AAV vectors displayed in vitro an integration frequency comparable to that reported for AAV vectors, and high transgene expression at a level comparable to the first-generation Ad vectors [163].

Baun's group did extensive studies exploiting the retrovirus integrating machinery to realize permanent Ad-delivered transgene expression. By flanking the transgene cassette in the Ad vector with the cis-acting elements from Moloney murine leukemia virus in a unique arrangement, they obtained high integration frequencies (between 5% and 11%) in rat spleen cells [165]. In consideration of the high transduction efficiency, this integration rate should be high enough to stably keep transgene expression at therapeutic levels for many gene therapy applications.

Kay and colleagues addressed the challenge of genome persistence by exploiting the promiscuous integration capabilities of the Sleeping Beauty (SB) transposon in Ad-mediated gene delivery [166]. SB transposon is a member of the Tc1/mariner superfamily of transposons, and undergoes cut-and-paste transposition through a DNA intermediate, mediated by the SB transposase [167]. By incorporating the SB integration machinery into an HD Ad vector containing human coagulation factor IX, therapeutic levels of human coagulation factor IX were sustained for more than 6 months in mice undergoing extensive liver proliferation [166].

However, at the current time there are great concerns about the risk of insertional mutagenesis due to random integration of vectors into the host genome. The recent clinical trial in France using

retroviral vectors in a gene therapy approach for X-linked severe combined immunodeficiency disease (X-SCID) had been taken as the milestone success in human gene therapy [168]. Unfortunately, two of the 11 patients treated during this X-SCID trial developed a leukemia-like disorder [169,170]. Now it is known that the cause of induced leukemia in X-SCID trial is attributed to the retrovirus vector integration in proximity to the LMO2 proto-oncogene promoter, which leads to aberrant transcription and expression of LMO2 [171]. Recent study suggests that the AAV vector preferentially integrates into active genes, though at present it is not clear if this will contribute to the risk of developing a malignancy [172]. Recchia's Ad/AAV hybrid vector system showed 35% of DNA integration was site-specific at the AAVS1 site in hepatoma cells, with one HD Ad vector expressing Rep78 and the second HD vector carrying a transgene flanked by AAV-ITRs [164]. However, excess Rep expression is toxic to the cells and may result in rearrangement of AAVS1 without transgene integration [173]. To avoid potential mutation risks of random integration, episomally replicating Ad vectors might be a potential alternative choice for long-term expression. Leblois et al. [174] inserted a loxP flanked Epstein-Barr virus (EBV) replicon into an Ad vector. Expression of Cre recombinase from a second Ad vector has been shown to release the transcription unit that could replicate coordinately during the cell cycle. Kreppel et al. [175] incorporated the episomally replicating machinery to an HD vector by utilizing the DNA recombinase FLPe to circularize the genome containing the EBV replicon, and obtained long-term transgene expression in proliferating cells. However, data of episomally replicating Ad vectors from in vivo studies are lacking.

4.7. Serotype switch or animal Ad vectors for readministration

In many gene therapy applications, lifelong expression might be needed, which would require readministration of vectors following the eventual loss of therapeutic transgene expression if the vectors have no integration or episomal replication machinery. However, readministration of an Ad vector will require the circumvention of the humoral immune response directed against the original vector capsid. Studies indicated that fiber-substituted Ad5 vectors containing fiber proteins of another serotype could not evade the humoral immune response against Ad5 [115], because hexon proteins of the capsid are the major targets of host-neutralizing antibodies in Ad5 infection [115,176,177]. This suggests that capsid partially modified Ad5 vectors do not meet the long-term need. Hence, the most practical approach to overcoming the obstacle of pre-existing antibodies is the use of alternative serotypes.

Morral et al. [178] observed in baboons that the hurdle of readministration due to the humoral response to an Ad5 vector was overcome by use of an Ad2 vector expressing hAAT. Their data further suggest that long-term expression of transgenes should be synergized by combining the reduced immunogenicity and toxicity of HD vectors with sequential delivery of vectors of different serotypes. However, it might be difficult to completely avoid cross-humoral response between Ad2 and Ad5 in all species including humans, as both Ads belong to the same subgroup and have high homogenicity. To address this issue, we and other groups have developed Ad35 vectors (subgroup B) [179–182] and have found that antibodies to Ad5 vector do not affect the transduction efficiency by Ad35 vector (Sakurai et al. unpublished observation, 181). Furthermore, while there is more than a 50% prevalence in adult humans of neutralizing antibodies to Ad5, less than 10% of individuals have anti-Ad35 neutralizing antibodies [181].

Since many humans are pre-exposed to human Ad, Ads from non-humans have been developed as vector systems for gene delivery. Mouse [183], avian [184], bovine [185], canine [186], porcine [187] and chimpanzee [188] Ad have been tested as vectors for various applications to overcome the barriers of pre-existing neutralizing antibodies. These animal-derived Ad vectors might be useful in evading humoral immune responses to human Ad5 for readministration. However, prior to their clinical application, safety issues such as oncogenicity should be addressed.

Readministration might be a solution to the transient transgene expression of Ad vectors when it is necessary. To balance the gain and loss in safety, efficacy and economy are the key points. The potential serotypes and animal origin Ads are limited.

Furthermore, this approach needs a complex set of clinical products.

5. Summary

Among all the vectors for gene delivery, no single one can meet all the requirements for all gene therapy applications. Similarly, for Ad vectors, no single approach can overcome the hurdle of immunogenic toxicities. It seems that HD vectors might serve as basis for combination with other approaches. It is clear that the ultimately ideal Ad vectors will have low or no immunogenic toxicities, and specifically transduce interested tissues/cells, with high, persistent and regulatable transgene expression. It is also clear that there is still a long way before research reaches this ideal.

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原　著

C 型肝炎ウイルス RNA の遺伝子検査法のための第一次国内標準品の作製

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ESTABLISHMENT OF THE FIRST NATIONAL STANDARD FOR NUCLEIC ACID AMPLIFICATION TECHNOLOGY ASSAY FOR HCV RNA

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The First WHO International Standard for HCV RNA for Nucleic Acid Amplification Technology (NAT) Assay (96/790) was established in 1997. The aim of our collaborative study was the establishment of the Japanese National Standard for HCV RNA calibrated against the WHO International Standard. The candidate materials were evaluated in the following two steps. First, titers of two HCV positive plasma (119 and 122) diluted in cryosupernatant were evaluated, and plasma 122 was chosen as the source plasma for the candidate for the national standard. Then, candidate 122 was prepared by diluting the source plasma to approximately 10^5 international units (IU)/m*l* in cryosupernatant. The relative potency of the candidate was measured against the International Standard by the end-point method. Seven laboratories from three countries participated in the collaborative study. Four laboratories used the Roche Amplicor assay (Version 1) and 3 laboratories used in-house PCR methods. There was reasonable agreement among the mean estimates from the laboratories. The overall mean potency of the candidate relative to the International Standard was $10^{5.00}$ ($10^{4.80}$～$10^{5.20}$) IU/m*l*. The sample was accepted as the first Japanese national standard and assigned a titer of 100,000 IU/m*l*. Each vial of the National Standard contains 0.5 m*l* of HCV plasma (genotype 1b) diluted in cryosupernatant and should be stored at−80℃.

Key words : HCV, The WHO International Standard, National Standard, Nucleic acid technology (NAT) assay, Blood safety

1. はじめに

供血者のC型肝炎ウイルス (HCV) に対する抗体スクリーニングを実施したにもかかわらずヨーロッパとアメリカ合衆国では血漿分画製剤によるHCVの感染が報告された．これは，HCVに感染してから抗体が検出されるまでのウインドウ期の血漿が原料血漿に混入していたためと考えられた[1)2)]．そこで，血液製剤のより一層のウイルス学的安全性の確保を目的としてヨーロッパでは1999年7月1日から原料血漿プールでHCV-RNAの核酸増幅検査 (NAT) を実施することになった．すでにイギリスをはじめオランダ，ドイツ，イタリア，アメリカ合衆国の各国では標準品やランコントロールを作製しており，NATを実施する施設で使用されていたが，HCV-RNA量がコピー数やgenome equivalent等まちまちの単位で表示されていたので，標準品のHCV-RNA量やNAT法の感度を相互に比較することが出来なかった．イギリスのNIBSCによってHCV-RNAの国際標準品作製のための国際共同研究が組織され，1997年10月にWHO国際標準品 (96/790) が制定され，国際単位を用いて各国参照品の力価を比較することが可能になった[3)4)]．わが国においては厚生省告示第427号によって，平成13年3月1日から製造され，又は輸入される血液製剤の原料血漿についてB型肝炎ウイルスDNA，C型肝炎ウイルスRNA及びヒト免疫不全ウイルスRNAに対するNATを実施しなければならないことに改められた．実際にはそれ以前に日本赤十字社の献血血液とすべての血漿分画製剤製造所の原料血漿プールについてHCV-RNAのNATが実施された．しかし，施設ごとにNAT法が異なり，自家標準品やキットの標準品の表示単位が統一されていなかったので，それぞれの施設での感度や精度を比較・評価することができなかった．国際単位で表示された広く認められた標準品を用いて感度や精度を測定することにより，施設間の比較や評価が可能になると考えられた．一方，国際標準品

はその配布数も限られており，国際標準品に対して較正された我が国独自の国内標準品の作製が望まれていた．そこで，血漿分画製剤の安全性確保対策の検討小委員会（以下，小委員会と略）はHCV-RNA量を国際単位で表示した国内標準品を作製するための共同研究を組織し，第一次HCV-RNA国内標準品を作製したので報告する．国際標準品はgenotype 1であるが，国内標準品は我が国で最も頻度の高いgenotype 1bとした．現在，さまざまなウイルスについて臨床や研究の場でNATが実施されているが，国内標準品として定められたものはまだない．その意味で，本標準品は我が国で初めて作製されたウイルスのNATのための国内標準品でもある．

2．材料および方法

1）国内標準品候補の原料血漿の選択

日本赤十字社より供与されたHBs抗原，抗HIV-1/2抗体，HBV-DNA，HIV-RNAのすべてが陰性で，HCV陽性の血漿の中から日本で最も高頻度に見られるgenotype 1bの2つの血漿（119と122）を標準品の原料候補とした．各原料血漿の一部を脱クリオプール血漿で約10^5国際単位（IU）/m*l*に希釈して－80℃で凍結・保存した試料を調製し，HCV-RNA国際標準品とともに参加施設に配布した．各施設は測定ごとに新しいバイアルの候補品を脱クリオ血漿で希釈して10倍希釈系列（10^{-1}から10^{-7}）を調製することとし，日を替えて2回定性的な方法でエンドポイントの測定を実施した．一重測定を原則としたが，日常的に二重測定を実施している場合は二重測定した（第1回測定）．このとき使用した国際標準品は小分けして－80℃に凍結保存して第2回測定に用いた．

2）HCV-RNA国内標準品候補の作製と評価

1）で選択した血漿122（PHA力価2^{14}，RNA量2～3×10^6IU/m*l*，容量185m*l*）をあらためて約10^5IU/m*l*に脱クリオ血漿で希釈，0.5m*l*ずつガラス瓶に分注し－80℃で凍結して，HCV-RNA国内参照品候補122（候補品）とし，参加施設に送付した．各施設は初回は10倍希釈系列で予備的なエンドポイントを測定し，より正確なエンドポイントの値を得るために2回目以降はそのエンドポイントをはさんで7段階の$10^{0.5}$希釈系列を測定ごとに調製し，日を替えて4回測定を実施した（第2回測定）．参加施設から返送された結果を集計して，HCV-RNA国内標準品候補のWHO国際標準品に対する力価を推定した．

3）参加施設と測定方法

日常的にHCV-NATを実施している9施設（国内6施設，米国2施設，ヨーロッパ1施設）に候補品を配布し，7施設（国内5施設，米国1施設，ヨーロッパ1施設）から試験結果が返送された．核酸の抽出と増幅の方法は各施設の任意の方法で実施した．

4）測定値の分析

候補品，国際標準品についてそれぞれのエンドポイント濃度の対数値の平均を求め，その比を国際標準品に対する候補品の対数相対力価とする．施設ごとに国際標準品に対する候補品の対数相対力価とその95％信頼区間を推定した．7施設から得られた対数相対力価の加重平均を求めて候補品の対数相対力価を推定した．対数相対力価の真数は国際標準品に対する候補品の相対力価を現すので，真数の値を国際標準品の力価に乗じて候補品の力価を推定した．

3．結　果

1）参加施設が実施した測定方法

血漿分画製剤製造所5施設（国内3，海外2），公的機関1施設，その他1施設の合計7施設から結果が返送された．Table 1に参加施設を表すコード番号，抽出法，検出法を示す．4施設がアンプリコアHCV（Ver. 1）変法，2施設が自家法のnested PCR法，1施設が自家法のsingle PCR法を用いて測定した．反応当たりの試料の量は40～400μ*l*の血漿に相当した．

2）原料血漿の選択

国内標準品は様々なNAT法に使用されるので，候補品にふさわしい原料を選択する目的で，第一回測定では2つのHCV陽性血漿119と122を希釈した試料を配布して測定した．大きな相違がなかったので，より多くの標準品の作製が可能なように容量の大きい血漿122を候補品の原料として選択した．血漿122のHCVコア領域の塩基

Table 1 Assays used in the collaborative study.

Laboratory	Assay	Extraction[a]	Eq. Vol. Amplified[b]
1	Amplicor	R&D	100
2	In-house single PCR	In-house NaI	40
3	Amplicor	Amplicor	50
4	Amplicor	R&D	100
5	Amplicor	QIAamp	400
6	In-house nested PCR	R&D	100
7	In-house nested PCR	R&D	100

a) R&D : Smi-test EX-R&D (Nippon Genetics Co. Ltd.)
Amplicor : Amplicor HCV version 1 (Roche)
QIAamp : QIAamp DNA Blood Mini Kit (QIAGEN)
b) Eq. Vol. Amplified : the equivalent volume of sample that was amplified in an assay

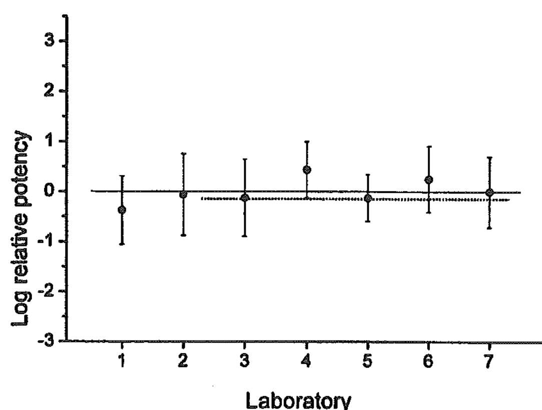

Fig. 1 Log relative potency of candidate 122 to the international standard (96/790). The laboratory code number and assay methods are explained in Table 1. The solid line indicates the mean log relative potency calculated from all data, −0.001 (−0.204-+0.201). The dotted line indicates the mean log relative potency calculated from the data excluding those of the laboratories 1 and 2, +0.066 (−0.161-+0.292).

Table 2 Estimated log potency of candidate 122 calibrated against the international standard (96/790). Overall (a) = the overall mean log potency calculated from all laboratories. Overall (b) = the overall mean log potency calculated from data excluding those of laboratories 1 and 2.

$\log_{10}$IU/m*l*

Laboratory	Mean	Minimum	Maximum
1	4.63	3.94	5.31
2	4.94	4.12	5.75
3	4.88	4.11	5.66
4	5.44	4.88	6.00
5	4.88	4.41	5.34
6	5.25	4.59	5.91
7	5.00	4.29	5.71
Overall (a)	5.00	4.80	5.20
Overall (b)	5.07	4.84	5.29

配列を決定して genotype 1b であることを確認した.

3）候補品 122 の国際標準品（96/790）に対する力価の推定

あらためて候補品を送付し，7 施設において $10^{0.5}$ 稀釈系列で測定した（第 2 回測定）．5 施設で独立の 4 回の測定，2 施設で各 2 回繰り返し測定を独立に 4 回行った．エンドポイント法により国際標準品に対する候補品の対数相対力価を求めた．なお，エンドポイントが最大希釈と同等となった場合は最大希釈をエンドポイントとした．また，不連続な陽性結果を含む場合は希釈率の高いほうをエンドポイントとした．施設毎に候補品の国際標準品に対する対数相対力価とその 95% 信頼区間を求め，全施設の測定結果を用いて候補品の国際標準品に対する対数相対力価を推定した．Fig. 1 に示すように全施設の結果は誤差の範囲で一致し，国際標準品に対する候補品の対数相対力価の平均は $\log 10^{-0.001}$ であった．WHO 国際標準品（96/790）の力価は 10^{5}IU/m*l* であるから，候補品の力価は $10^{5.00}$（$10^{4.80\sim5.20}$）IU/m*l*，即ち 100,000IU/m*l*

と推定された（Table 2）.

参加7施設中，施設1では測定4回中3回でエンドポイントが最大希釈と同等となった．また施設2では不連続な陽性結果が多く，測定結果のばらつきが大きかった．そこで，この2施設を除く5施設の測定結果を用いて分析した結果，5施設の結果は誤差の範囲で一致し，国際標準品に対する候補品の対数相対力価の平均は $\log 10^{0.066}$ であった（Fig. 1）．よって，候補品の力価は $10^{5.07}$（$10^{4.84 \sim 5.29}$）IU/m*l*，即ち116,300IU/m*l* と推定され，全施設の結果を用いた分析結果と有意な相違は認められなかった（Table 2）．最尤法で本研究の測定値を分析すると候補品の推定力価は $10^{5.07}$（$10^{4.86 \sim 5.30}$）IU/m*l* となり，2つの分析法による推定値はよく一致した．

以上の結果から，候補品122の国際標準品に対する力価は $10^{5.00}$IU/m*l* と推定され，力価100,000 IU/m*l* の国内標準品として1999年12月に小委員会で承認された．

4. 考　察

一般に個々の施設で国際標準品に対する2次標準品を作製すると新たな誤差が生じるので，異なる2次標準品を用いて測定した結果を相互に比較するのは困難である．HCV-RNA NAT試験において異なる施設間での測定値の比較や施設毎の検出感度の管理を実施するためには性状が詳しく調べてある広く認められた共通の標準品が必須である．本共同研究によってわが国で初めて，国際単位表示されたHCV-RNAの国内標準品が制定された．候補品の95% 信頼区間は力価 $10^{5.00}$IU/m*l* に対して $10^{4.80 \sim 5.20}$IU/m*l* であった．また参加施設のなかの力価の最大は $10^{5.44}$IU/m*l*（施設4），最小は $10^{4.63}$IU/m*l*（施設1）で $10^{0.81}$ 倍の相違であった（Table 2）．これらの値はエンドポイントの測定を $10^{0.5}$ 倍希釈系列で実施したことを考慮すると十分に小さいといえる．これは本共同研究の参加施設を日常的にHCV-NATを実施している信頼性の高い施設に限ったためと考えられる．国内標準品は分与される予定であるので，血液製剤の安全性確保のためのNAT試験法や診断薬の評価，臨床検査センターにおけるHCV-RNA検査の評価に広く用いられるようになれば，相互の性能を容易に比較することが可能になり，試験法・検査技術の向上が期待できる．各施設で国内標準品を用いて繰り返し測定することにより有効検出限界の推定値を得ることが可能である．こうして得られた有効検出限界をもとに，たとえば95% 陽性反応を得られる濃度と50% 陽性反応を得られる濃度の標準品を常に測定に加えた測定結果を集積し，継続的に各試験法の感度管理の精度向上を図ることが望まれる．

5. 結　論

血漿のHCV-RNAのNATのための国内標準品を作製した．国内標準品はHCV抗体陽性のHCV genotype 1b陽性血漿を脱クリオ血漿で希釈し，0.5m*l* ずつバイアルに分注，−80℃で凍結保存したもので，その力価は100,000IU/m*l* である．

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