智一郎, 普後一, 濱野國勝,宮村達男, 武田直和, 片山和彦:サポウイルス粒子形成機構の解析. 第52 回 日本ウイルス学会学術集会, 横浜, 2004 年 11 月 21 日.

(12) Hansman Grant, 名取克郎, 岡智一郎, 小川智子, 牛島廣治, 武田直和, 片山和彦: Cross-reactivity among sapovirus recombinant capsid proteins. 第52 回 日本ウイルス学会学術集会, 横浜, 2004 年 11 月 21 日.

H. 知的財産権の出願、登録状況

1. 特許取得　　なし。
2. 実用新案登録 なし。
3. その他　　なし。

ウイルス科の SaV は小児散発性非細菌性下痢症の原因ウイルスの一つであるがそのレセプター分子は明らかにされておらず、型物質との結合についても報告がない。そこで、NoV の GI に属する 4 株、GII に属する 10 株の計 14 株の NoV VLPs、さらに SaV GI の 1 株の VLPs を用い、型物質との結合パターンを検討した。

B. 研究方法

型物質は赤血球以外にも腸管上皮細胞などに発現されており、さらに唾液中にも分泌されている。今回、唾液中の型物質とウイルスとの結合を解析した。

1. 唾液中の型物質の定量

日本人である健常人 20 歳代から 60 歳代の成人男女 29 人から採取した唾液を用いた。採取後、直ちに 100℃、10 分間加熱処理を行い、その後 13,000g、5 分間にて遠心し、その上清を回収した。上清中の H、A、B、Le^a、Le^b 各型物質量を分泌型試験の半定量系により測定した。唾液の 2 階段希釈を行い、256 倍まで希釈した。抗 H レクチン、抗 A 抗体、抗 B 抗体（以上 gamma Biologicals, Inc., Huston, TX）、抗 Le^a 抗体、抗 Le^b 抗体（以上 Ortho-Clinical Diagnostics, Inc., Raritan, NJ）それぞれ 100μl、50μl、50μl、50μl、50μl と等量の唾液サンプル（原液と各希釈倍率のサンプル）を 26℃で 10 または 20 分間反応させた後に、50μl の O 型赤血球、A1 型赤血球、B 型赤血球（以上 gamma Biologicals, Inc.）、Ficin 処理 Le^a 陽性赤血球、Ficin 処理 Le^b 陽性赤血球（以上 Ortho-Clinical Diagnostics, Inc.）を加え 26℃で 5 分間インキュベーションを行った。125g、1 分間にて遠心後、穏やかに振トウし、肉眼的検査により凝集阻止の有無を調べた。

2. 唾液と VLPs との結合量の測定

唾液と VLPs との結合量を Saliva-VLP binding assay (ELISA-based) にて検出した。加熱、遠心後の唾液上清サンプルを 50 mmol/L, pH 9.6, carbonate/bicarbonate buffer にて 1600 倍に希釈し、Polystyrene plate（Thermo Labsystems）に加え、37℃で一晩コーティングを行った。5% skim milk(SM)/PBS にて、室温で 1 時間ブロッキングを行った後に、rNoV VLPs（0.5 μg/mL in 1% SM/PBS-T（0.05% Tween 20/PBS））を加え、37℃で 1 時間インキュベーションした。次いで rabbit anti-rNoV VLPs antiserum（at 1/2000 in 1% SM）を加え 37℃で 1 時間、horseradish peroxidase（HRP）-conjugated anti-rabbit IgG（Zymed Laboratories Inc.）を加え 37℃で 1 時間

インキュベーションを行った。O-phenylenediamine（Sigma）を基質として加え発色させた後に、492 nm にて吸光度を測定した。

（倫理面への配慮）

唾液の使用は、国立感染症研究所医学研究倫理審査委員会での承認、提供者からのインフォームド・コンセントを得た上で行った。

C. 研究成果

1. 唾液中の型物質の検出

型物質分泌パターンは以下のようになった： Secretor(Se)/A/Le(a-b+) 4 検体、Se/A/Le(a-b-) 1 検体、Se/AB/Le(a-b+) 6 検体、Se/AB/Le(a-b-) 2 検体、Se/B/Le(a-b+) 5 検体、Se/B/Le(a-b-) 1 検体、Se/O/Le(a-b+) 4 検体、Se/O/Le(a-b-) 1 検体、Non-secreotr/Le(a+b+) 5 検体。全体で Le(a-b+)、Le(a-b-)、Le(a+b+)表現型の比率はそれぞれ 66%、17%、17%であり、この値は日本人における比率 73%、10%、17%に大体一致した。A、AB、B、O 型別はすべての Secretor 検体で赤血球上の血液型（自己申告）と一致した。

2. 唾液と VLPs との結合量の測定

NoV GI の VLPs はすべて唾液と結合した。GI/1 クラスターのウイルス株は H または A 型物質量の多い唾液、GI/2、3、4 クラスターの株は H、A、Le^a 型物質のうちいずれかの量が多い唾液への結合が高かった。クラスターによって結合パターンに違いはあるものの、GI では共通して H、A 型物質に結合しやすいことが明らかとなった。NoV GII の VLPs は 7 株が唾液に結合し、3 株は結合しなかった。同じクラスターに属するウイルス株は同じ結合パターンを示した。GII/4 に属する株は、H、A、B、Le^a 型物質のいずれかの量が多い唾液、GII/6 は、H、A、B 型物質のいずれかの量が多い唾液、GII/3、5 は、A、B 型物質どちらかの量が多い唾液への結合が高かった。GII/12 は B 型物質を含む唾液に結合した。以上のことにより、GII では共通して A、B 型物質に結合しやすいことが明らかとなった。また、GII/1 の 2 株、GII/14 の 1 株は唾液に結合しなかった。SaV GI/1 に属する 1 株も唾液に結合しなかった。

D. 考察と結論

カリシウイルス科 *Lagovirus* 属のウサギ出血病ウイルスは H 型物質に結合することが知られている。しかし、今回の研究で、NoV の型物質認識パターンは様々であり、型物質を認識しない株も存在することが明らかになった。また、SaV は型物質に結合しない

可能性があり、カリシウイルス科に共通の特徴ではないことが示唆された。今後さらに詳細な解析を行うことにより、型物質がカリシウイルスの単なる結合因子として働いているのか、細胞への侵入にも関与するレセプターであるのかを検討したい。

D. 研究発表

1. 誌上発表

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(2) 武田直和、米山徹夫、清水博之、白土（堀越）東子：食品由来の感染症．ネオエカス感染症・アレルギーと生体防御　印刷中

(3) 白土（堀越）東子：ノロウイルスによる感染性胃腸炎の集団発生．生活と環境　印刷中

2. 学会発表

(1) 白土（堀越）東子、名取克郎、小川智子、Hansman Grant、鎌田公仁夫、影山努、片山和彦、宮村達男、武田直和「カリシウイルスと血液型物質との結合の解析」第52回日本ウイルス学会学術集会、2004年11月21-23日、横浜

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カリシウイルスデータベース構築に関する報告

分担研究者；国立感染症研究所ウイルス第二部　片山和彦
研究協力者；札幌医科大学医学部　三瀬 敬治

カリシウイルス科には、ラゴウイルス、ベジウイルス、ノロウイルス、サポウイルスの４つのウイルス属が存在する。人に感染するのはノロウイルスとサポウイルスである。近年、ノロウイルスについては、複数の株のゲノム全長塩基配列が明らかにされるとともに分子遺伝学的解析が進み、ゲノタイピング法が確立された。最新の論文（JCM vol.42, p2988-2995, 2004, Kageyama T., et al)によれば、ノロウイルスはGI, GII二つのゲノグループに大別され、GIには14のゲノタイプ、GIIには17のゲノタイプが確認されている。サポウイルスについては、ノロウイルスの5～8年あまり昔を後追いしている状況である。現在、幾つかのグループによってサポウイルスの塩基配列データの蓄積が行われつつある。今後、遺伝子解析が進み、VLPsの発現や抗原性など基礎的な分野ばかりでなく、分子疫学の進展が期待できる。また、研究の進展に伴い、ノロウイルスやサポウイルスはゲノムの非構造蛋白質コード領域と構造蛋白質コード領域の境界でゲノムの組換え（リコンビネーション）を起こして、劇的に進化することが明らかになってきた。さらに、ノロウイルス、サポウイルスにはウシやブタから発見され、ゲノム塩基配列が明らかにされた株も存在している。ヒトのウイルスと動物のウイルスの間でゲノムの組換えが起きる可能性もあり、人獣共通感染症として考える必要があるかもしれない。

このような研究を支えているのは、世界3大遺伝子データベースとして知られているDDBJ, Genbank, EMBLである。DDBJには月当たり３億塩基を超える登録が行われており、年々登録数も増え続けている。遺伝子データベースはいわば、遺伝情報の海を通り越して宇宙であると言っても過言ではない。このように広大な宇宙から、カリシウイルスの研究者が必要とする情報を引き出すのは容易なことではない。そこで、カリシウイルスに特化したデータベースを作成し、DDBJと協力しながらカリシウイルス研究者の遺伝子配列情報の解析をサポートすることを目的としたサブデータベース及び情報交換の場の構築を試みた。

ホームページ上では、ノロウイルスの分子系統解析法を簡単に習得し、実行できるようにガイドした“分子系統樹の作り方”（パワーポイントファイル）を公開した。また、ノロウイルスの各ゲノタイプの標準配列を公開し、ホームページにアクセスした研究者が、自由にダウンロードして分子系統解析に使用できるようにした。さらに、研究者間のコミュニケーションが図れるように掲示板形式のフォーラムサイトをもうけ、ホームページ上で意見交換ができるようにした。

今後、カリシウイルスデータベースに希望者が自由にアクセスし、十分に活用できるようにページデザインの見直し、登録データの充実などを図っていく予定である。

カリシウイルスの分子疫学

分子系統樹の作り方

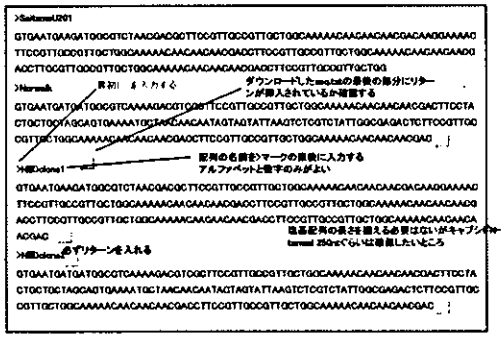
分子系統解析の進め方（アウトライン）

- CapsI領域のprimer set
  - Norovirus: G1SKF & G1SKR, G2SKF & G2SKRを用いたRT-PCRで増幅したPCR産物の塩基配列を用いる（primer部分は塩基配列データから削除する）のが良い。この部分の配列であればどのようにしてまめても良い。
  - Sapovirus: 現在推奨するprimer setは無いが、capsid領域のN末端300ntほどを用いると良い。後に推奨するプライマーセットを策定する予定。
- ホームページ上にあるNorovirus seq file, Sapovirus seq file(掲載予定) に新規塩基配列を貼り付けて保存する。
- Clustal Wでアライメントすると共に、distanceの算出をする。
- Clustal Wで得られたphbファイルをワープロで開き、text (pure text) として保存する。
- NJ plotまたはTreeViewを起動し、phb.txtを読み込み分子系統樹を作製する。
- この系統樹に含まれる標準株を指標にgenotypeクラスターを分けてgenotypeを決定する。
- 対象株が新しいクラスターを形成した場合、Bootstrap（分岐点検定）で樹形の信頼度を評価する。
- Clustal Wで得られたphylip distance fileを用いて新しいクラスターのdistanceをチェックする。DDBJに塩基配列を登録する。

Step1. 塩基配列の入力と保存

- Norovirus seq file, Sapovirus seq fileをダウンロードし、解析したい配列を加え、テキスト(.txt)で保存する。
- 方法の詳細、注意点は次のページに示した。
- >マークを入力し、直後に塩基配列名を入力する。
- リターンを入力した後、塩基配列をコピー＆ペーストする。
- 再びリターンを入力する。
- NVseq1など適当な名前を付けテキストフォーマットで保存する（Pure text, RLFはだめ）。

Norovirus seq fileへのsequence dataの追加方法



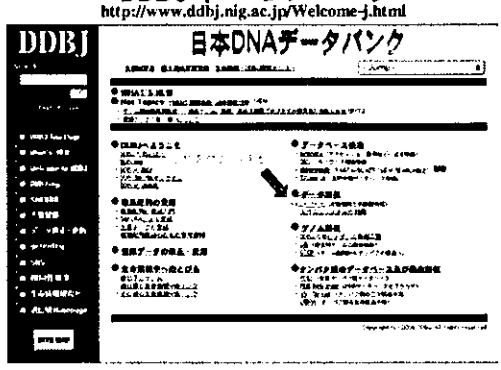
保存は必ずテキストフォーマットで、例えば Notepad などで

Step2. Clustal Wへの送信

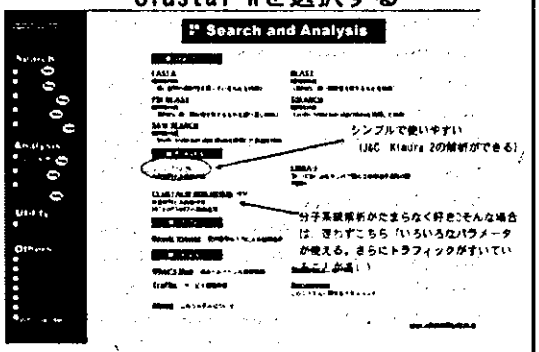
- DDBJ home page (http://www.ddbj.nig.ac.jp/Welcome-j.html) に行く
- テキスト（name.txt）で保存したシーケンスファイルをDDBJホームページのClustal Wに送信する
- 解析の諸条件を入力する
- メールアドレスを入力する
- 送信ボタンを押す
- 返事が戻ってくるのを待つ

DDBJホームページ

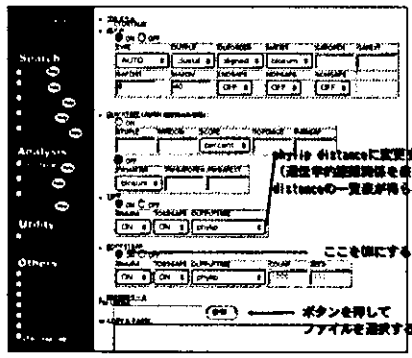
http://www.ddbj.nig.ac.jp/Welcome-j.html



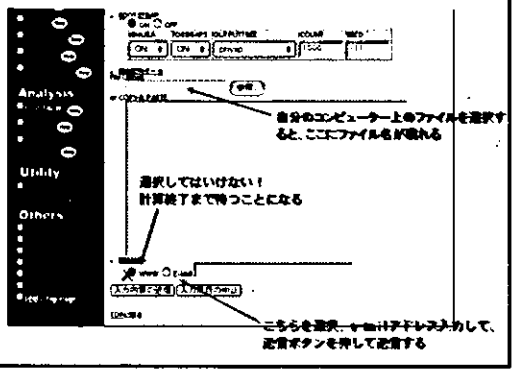
Clustal Wを選択する



Clustal Wのパラメータは標準のままでよい (ここで示した場所のみ変更する)



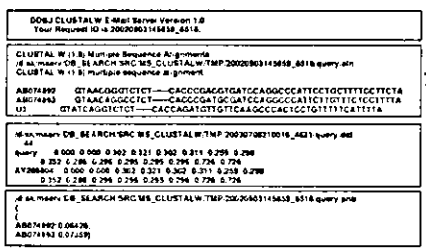
塩基配列ファイルを送信する



Step3. Clustal W dataの利用

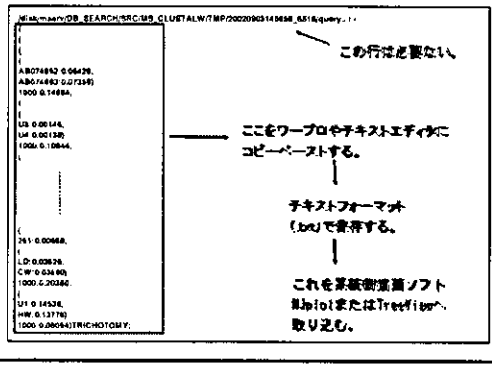
- メールボックスに送信されてきたデーターを解読する。
- bootstrap tree fileをコピーペーストでテキストエディタに運ぶ。
- bootstrap tree fileに適当な名前を付け、テキストで保存する。
- Distanceのヒストグラム解析が必要ならdistance matrix fileを統計解析用のソフトに転送し、解析を行う。

Clustal W dataの利用方法



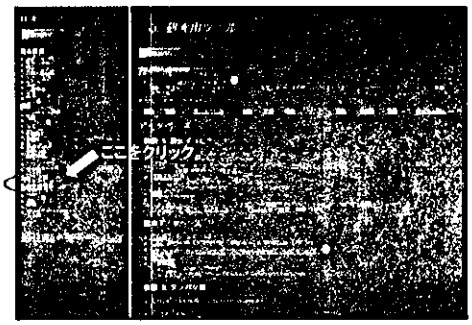
解析結果が上記のように分かれている。それぞれの結果の上部に付くヘッダーを目印にし、必要な部分をテキストエディターにコピーペーストする。

コピーペーストする部分；目印は．phb

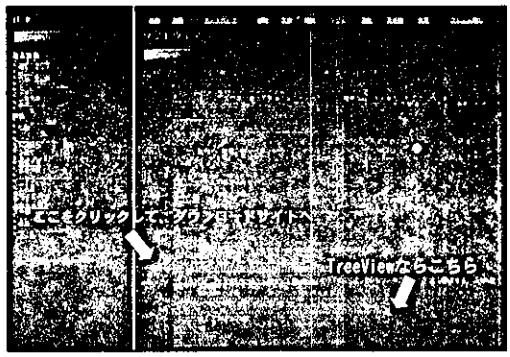


NJplotまたはTreeViewをダウンロードする

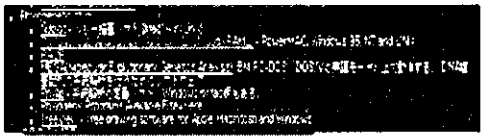
QUADROPHENIAに行き、ダウンロードサイトへ向かう
(http://www.nih.go.jp/~jun/research/index-j.html)



分子系統解析のための秀悦なソフトたち



Njplot & TreeView

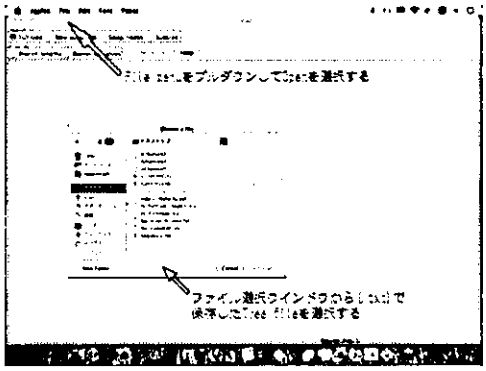


- NJplotはすべてのプラットフォームで使用可能
  (NJを描ける unroot, rootどちらでも)
- TreeViewはマック又はウィンドウズ (NJ, UPGMAなど多様なtreeを描ける)
- 両ソフトともにフリーウェア!
- 系統樹を画像で保存して使用可能

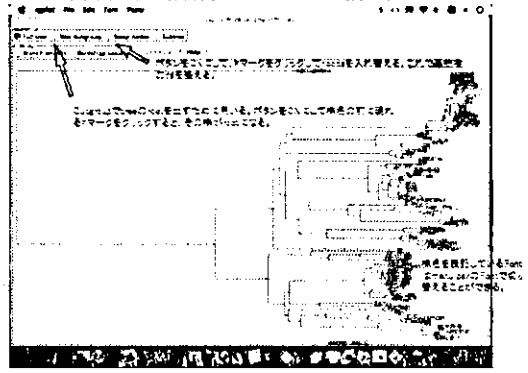
Step5. NJplotで系統樹を描く

- NJplotアイコンをダブルクリックして起動する。
- File menuからopenを選択する。
- (.txt) で保存したtree fileを選択する。
- メインウインドウに分子系統樹が現れるハズ
- 分子系統樹を加工する。

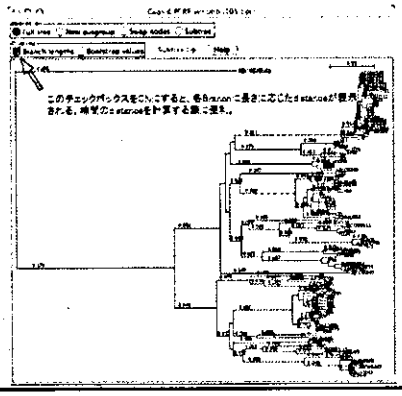
NJplotの起動画面



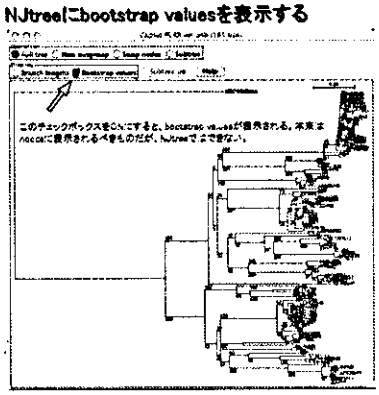
読み込みに成功するとNJtreeが描かれる



NJtreeにdistanceを表示する



NJtreeにbootstrap valuesを表示する



Step6. NJtreeを出力する

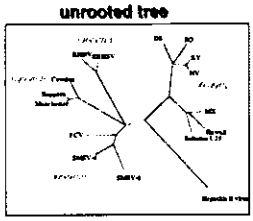
- File menuのSave plotを選択すると、NJtreeが.(.pict)で保存できる(Macの場合)。このファイルはApple works, Adobe Illustrator等で加工して利用できる。
- Windowsの場合、同様にSave plotを選択すると.psfというポストスクリプトファイルで保存される。これを利用する。

クラスタリング

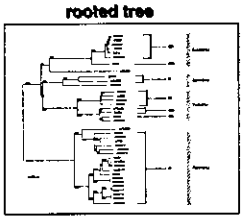
- Webからダウンロードした塩基配列データには各ジェノタイプの標準株が入っている。この株を目安にgenotypeクラスターを特定する。
- 解析している塩基配列が新しいクラスターを形成したなら、bootstrap valueと近縁のクラスターからのdistanceを確認する。
- できればdistanceの分散分析を行い、genotype distanceの3SDに入る場合、新たなgenotypeとしてナンバリングし、DDBJに登録する。
- 詳細はkageyamaら、JCM, 2004 in pressを参照

Neighbor - Joining (NJ)の特徴

unrooted tree



rooted tree



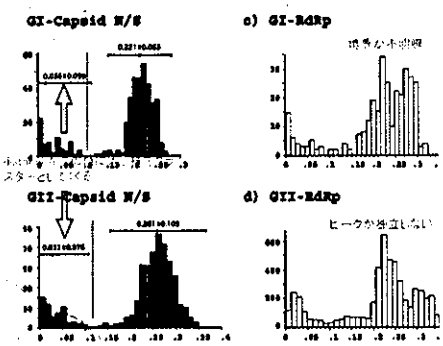
- 最も近しい関係を順次結合する方法。
- 進化速度が一定でない場合でも仕様可(ウイルスには良い)。
- 無根系統樹(rootを設定するにはout groupが必要)。
- 無根であるため種の分化様式を見いだしにくい。
- クラスタリングが難しい。

Phylip Distanceの利用方法

	A	B	C	D	E	F	G	H
A	0.000	0.000	0.302	0.321	0.302	0.311	0.259	0.298
B	0.000	0.000	0.302	0.321	0.302	0.311	0.259	0.298
C	0.302	0.302	0.000	0.013	0.019	0.006	0.416	0.441
D	0.321	0.321	0.013	0.000	0.019	0.006	0.439	0.46
E	0.302	0.302	0.019	0.019	0.000	0.013	0.416	0.441
F	0.311	0.311	0.006	0.006	0.013	0.000	0.428	0.454
G	0.259	0.259	0.416	0.439	0.416	0.428	0.000	0.026
H	0.298	0.298	0.441	0.465	0.441	0.454	0.026	0.000

- phylip distanceは、このような表の形になっている。
- 横軸にdistanceをとり、縦軸に出現頻度(何個同じ数字があったか)をとって、棒グラフを書く(ヒストグラム)
- 対角線にZEROが並んでいる。この対角線に対して線対称に数値が並んでいるので、利用するのはどちらか一方でよい。消去が面倒な場合は、出現頻度を計算してから、頻度を2で割ればよい。

ヒストグラムを作りクラスタリングの基準を作る



Windows XPの場合はTreeViewが良いかもしれない

DDBJ homepageのclustal Wのページの右側のカラムにclustal W Helpがある。

これをクリックすると、TreeViewとclustal Wの使い方が詳細に記載されている。

分子疫学を究めたいならMEGAの使用をお勧めする。
http://www.megasoftware.net/よりフリーで入手可能。
詳細なマニュアルも用意されており、多機能。ただし、日本語をサポートしていない。

おわり

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Isolation of Human Monoclonal Antibodies That Neutralize Human Rotavirus

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A human antibody library constructed by utilizing a phage display system was used for the isolation of human antibodies with neutralizing activity specific for human rotavirus. In the library, the Fab form of an antibody fused to truncated cp3 is expressed on the phage surface. Purified virions of strain KU (G1 serotype and P[8] genotype) were used as antigen. Twelve different clones were isolated. Based on their amino acid sequences, they were classified into three groups. Three representative clones—1-2H, 2-3E, and 2-11G—were characterized. Enzyme-linked immunosorbent assay with virus-like particles (VLP-VP2/6 and VLP-VP2/6/7) and recombinant VP4 protein produced from baculovirus recombinants indicated that 1-2H and 2-3E bind to VP4 and that 2-11G binds to VP7. The neutralization epitope recognized by each of the three human antibodies might be human specific, since all of the antigenic mutants resistant to mouse monoclonal neutralizing antibodies previously prepared were neutralized by the human antibodies obtained here. After conversion from the Fab form of an antibody into immunoglobulin G1, the neutralizing activities of these three clones toward various human rotavirus strains were examined. The 1-2H antibody exhibited neutralizing activity toward human rotaviruses with either the P[4] or P[8] genotype. Similarly, the 2-3E antibody showed cross-reactivity against HRVs with the P[6], as well as the P[8] genotype. In contrast, the 2-11G antibody neutralized only human rotaviruses with the G1 serotype. The concentration of antibodies required for 50% neutralization ranged from 0.8 to 20 μg/ml.

Rotavirus is the major cause of severe acute gastroenteritis among infants and young children. Rotavirus infection is life-threatening in developing countries, resulting in 500,000 to 600,000 deaths annually (33). In developed countries, rotavirus infections lead to a high disease burden with considerable medical expense due to the high morbidity. Furthermore, adults, particularly the elderly, are also affected by rotavirus infection (34, 39), and immunocompromised children and adults develop persistent rotavirus diarrhea (12, 42, 43). Thus, vaccination is thought to be the best way to reduce severe rotavirus gastroenteritis worldwide. Tetravalent rhesus rotavirus (RRV) human reassortant vaccine comprising RRV and three RRV-based monoreassortants carrying the VP7 genes from G1, G2, and G4 human rotaviruses (HRVs) was developed (25), and 1.5 million doses of this vaccine had been administered to infants by the end of May 1999 in the United States. However, the vaccine was withdrawn due to the occurrence of gut intussusception, which appeared to be epidemiologically linked to vaccine application (5, 38). Moreover, even if a safe and effective rotavirus vaccine is developed, vaccination would be less effective in immunocompromised patients.

Rotaviruses have two outer capsid proteins, viral protein 4 (VP4) and VP7, encoded on RNA segment 4 and RNA segment 7, 8, or 9, depending on the strain, respectively (19). VP4 and VP7 are known to induce neutralizing antibodies (Abs) in the sera and stools of infected patients, and they are relevant to protection against rotavirus infection (14, 18, 41, 45–47, 51). It is well known that the rotavirus G serotypes and P genotypes defined by VP7 and VP4, respectively, exhibit diversity. A total of 15 G serotypes and 22 P genotypes have been described (11). Although the majority of HRVs prevailing worldwide have G1, G2, G3, or G4 as the G serotype, and P[4] or P[8] as the P genotype, at least 10 G and 10 P types have been reported on HRVs (8). Recently, a number of HRV strains with unusual G or P types and rare combinations of G and P types have been detected worldwide. For example, G9 is increasing rapidly. In contrast, both VP4 and VP7 carry heterotypic (cross-reactive) neutralization epitopes, which are thought to be related to heterotypic protection (29, 30, 45–47, 49). An individual can be repeatedly infected with various strains of HRVs, suggesting that he or she has broadly and strongly effective Abs to HRVs. Although the validity of passive immunization remains unclear (17), oral administration of cross-reactive human immunoglobulins could be one of the measures for both prophylaxis and therapy for HRV diseases.

The natural repertoire formed in the human body should be composed of two different types of Abs. One type, which forms a naive repertoire, should show a wide range of antigen (Ag) specificity, the Ab binding avidity of each Ab being low in general. The other type, which is raised against specific Ags by immunization, should show a narrow range of specificity, the Ab binding avidity of each Ab being strong. In the present study, we used the Ab library called AIMS4 constructed from the B lymphocyte-rich tissues of a few dozen patients. Since this library is human-derived and rotavirus infection is considered to be very common worldwide, we expected that the Ab

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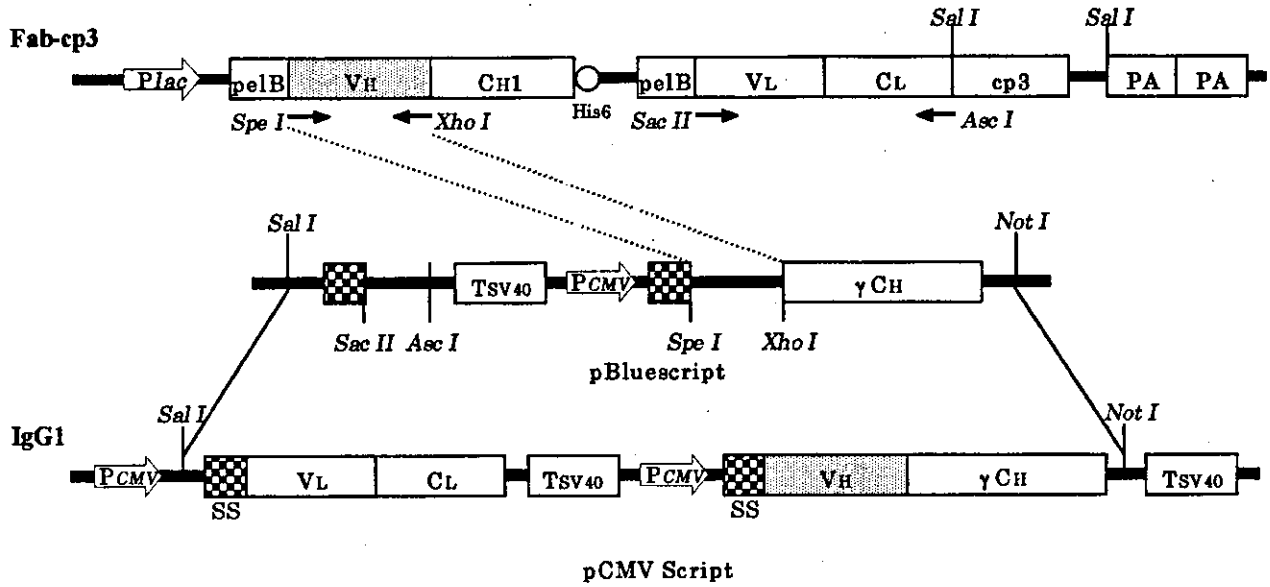

FIG. 1. Scheme for the conversion of a phage Ab (Fab-cp3) to an human IgG1. The V_H and the V_L C_L regions were amplified by PCR with the proper primers listed in Table 1, followed by subcloning into the proper restriction enzyme sites to construct an IgG1 cassette. The IgG1 cassette was then cloned into expression vector pCMVScript. *Plac*, *lac* promoter; *pel*B, *pel*B leader sequence; C_H1, the first H-chain constant domain of human IgG1; His6, His tag-encoding part; cp3, truncated cp3; PA, Fc-binding domain of protein A; Tsv40, simian virus 40 terminator; P_{CMV}, cytomegalovirus promoter; γC_H, human γC_H domain; SS, signal sequence.

repertoire formed in AIMS4 should reflect a variety of rotavirus-specific Abs acquired through natural exposure. It would be interesting to directly explore the Ab repertoire of humans. In particular, comparison of the neutralization epitopes recognized by humans and mice would be useful for understanding the immune response against rotavirus infection in humans.

We describe here the successful isolation of anti-VP4 cross-reactive Abs and an anti-VP7 G1-specific Ab with neutralizing activities toward rotaviruses.

MATERIALS AND METHODS

Viruses. The following HRV strains and reassortants were used for the present study: KU (G1P[8]), Wa (G1P[8]), M37 (G1P[6]), K8 (G1P[9]), S2 (G2P[4]), 1076 (G2P[6]), YO (G3P[8]), McN13 (G3P[6]), AU-1 (G3P[9]), Hosokawa (G4P[8]), 69M (G8P[10]), WI61 (G9P[8]), L26 (G12P[4]), and two bovine strain UK-based single gene-reassortants, UK/Wa (G1P[5]) and UK/DS1 (G2P[5]), carrying the VP7 gene from HRV strains Wa and DS1, respectively (32). Eleven antigenic KU mutants resistant to each of 11 neutralizing mouse monoclonal Abs (MAbs) were also used in the present study: six (V-YO-1E6, V-ST-1F2, V-YO-1S3, V-YO-2C2, V-KU-4D7, and V-KU-6B11) and five (V-KU-3C7, V-YO-4C2, V-KU-5H1, V-KU-6A11, and V-KU4) mutants have been prepared by cultivating strain KU in the presence of anti-VP4 cross-reactive neutralizing MAbs and anti-VP7 G1-specific neutralizing MAbs, respectively (45, 47). Virus propagation and purification were carried out as described previously (49). Unless otherwise stated, the culture fluids of MA104 cells infected with rotaviruses were used for the assays.

Preparation of virus-like particles (VLPs) and recombinant VP4. Construction of the artificial VLP of HRV KU origin is described elsewhere (K. Taniguchi et al., unpublished data). Briefly, the reverse transcription-PCR products of the VP2, VP4, VP6, and VP7 genes of human strain KU were cloned into a TA cloning vector, pCRII (Invitrogen, San Diego, Calif.), to generate pKU-VP2, pKU-VP4, pKU-VP6, and pKU-VP7. After digestion with restriction enzymes, the fragments were ligated into transfer vector pVL1392 to yield pVL1392/KU-VP2, -VP4, -VP6, and -VP7. Sf9 cells were coinfected with linearized wild-type *Autographa californica* nuclear polyhedrosis virus DNA (Pharmingen) and either pVL1392/KU-VP2, -VP4, -VP6, or -VP7 by the Lipofectin-mediated method. The baculovirus recombinants thus obtained were used for preparation of recombinant VP4, VLP-VP2/6, and VLP-VP2/6/7 in Tn5 cells.

Ab library. Abs were isolated from the Abs library called AIMS4, which was constructed in Y. Kurosawa's laboratory. In brief, B lymphocyte-rich fractions of human tissues such as tonsils, umbilical cord blood, peripheral blood, and bone marrow were used as gene sources of Abs (35; Y. Akahori et al., unpublished data). Using a phage-display system, the Fab form of an Ab fused to a truncated cp3 (Fab-cp3) was expressed on the phage surface. The library is composed of 10^{11} independently established clones, and it has been shown that >70% of the phages express Abs.

Screening of the library. Selection of phages exhibiting rotavirus (strain KU)-binding activity was performed by a panning method that was essentially the same as that described previously (20, 27). The immunotubes (Nunc-Immunomodules Polysorp) were coated with 200 μg of a purified KU virion/ml in phosphate-buffered saline containing 100 μg of Ca^{2+} and Mg^{2+}/ml [PBS(+)] overnight at 4°C. After a blocking step with 2% skim milk, a solution of phages (10^{14} CFU) was added to each tube, followed by incubation at room temperature for 2 h. The unbound clones were washed out four times with PBS(+). Bound phages were eluted with 0.1 M triethylamine (pH 12.3), and the eluent was then immediately neutralized with 1 M Tris-HCl (pH 6.8). *E. coli* DH12S cells cultured in 2xYT medium were infected with the eluted phages, precipitated by centrifugation, and then resuspended in 2xYT containing 1% glucose and 100 μg of ampicillin/ml, followed by superinfection with helper phages and further cultivation under kanamycin-selective conditions (70 μg/ml) in order to replicate phage clones harboring KU-reactive Abs. The phage clones obtained through this process were used for the next round of panning. The input titers of the phages and the number of washings with PBS(+) were 1.06×10^{13} and 8 for the second panning and 3.46×10^{13} and 16 for the third panning, respectively. After the third round of panning, DH12S cells infected with the selected phages were spread on LB plates containing 1% glucose and 100 μg of ampicillin/ml and incubated at 30°C overnight.

Preparation of various forms of Abs. The individual clones of *E. coli* infected with phages were grown in 2xYT medium containing 0.1% glucose and 100 μg of ampicillin/ml. After the addition of 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside), the Fab-cp3 molecules were initially accumulated in the periplasm of *E. coli* and then gradually secreted and/or released into the culture medium (crude Fab-cp3). On average, 1 μg of Fab-cp3 molecules/ml is present in the culture fluid. The Fab-cp3 molecules can be purified with anti-cp3 MAb-conjugated Sepharose beads. After isolation of phage particles, the gene encoding an Fab-cp3 molecule can be easily converted into another gene encoding an Fab-PP (P denotes a single Fc-binding domain of protein A) form of Ab by digestion with SalI followed by self-ligation (Fig. 1) (22). The Fab-PP molecules can be purified

TABLE 1. Oligonucleotide primers used for the conversion of phage antibodies (Fab-cp3) to human IgG1

Primer	Orientation[a]	Sequence
1-2HVH	F	5′-TTCCTCCTACTAGTGGCAGCTCCCAGATGGGTCCTGTCCCAGGTGCAGCTGGTGCAGTCTGG-3′
	R	5′-GGTGGAGGCACTCGAGACGGTGACCAGGGTTC-3′
1-2HVLCL	F	5′-CTACTCTGGCTCCGCGGTGCCAGACAGTCTGTGTTGACGCAGCCG-3′
	R	5′-TCGACTGGCGCGCCCTATGAACATTCTGTAGGGGCCACTGTCTTC-3′
2-3EVH	F	5′-TTCCTCCTACTAGTGGCAGCTCCCAGATGGGTCCTGTCCGAGGTGCAGCTGGTGGAGTCTGG-3′
	R	5′-GGTGGAGGCACTCGAGACGGTGACCATTGTCC-3′
2-3EVLCL	F	5′-CTACTCTGGCTCCGCGGTGCCAGAGAAACGACACTCACGCAGTCT-3′
	R	5′-TCGACTGGCGCGCCCTAACACTCTCCCCTGTTGAAGCTCTTTGTG-3′
2-11GVH	F	5′-TTCCTCCTACTAGTGGCAGCTCCCAGATGGGTCCTGTCCGAGGTGCAGCTGGTGGAGTCTGG-3′
	R	5′-GGTGGAGGCACTCGAGACGGTGACCGTGGTCC-3′
2-11GVLCL	F	5′-CTACTCTGGCTCCGCGGTGCCAGAGAAACGACACTCACGCAGTCT-3′
	R	5′-TCGACTGGCGCGCCCTAACACTCTCCCCTGTTGAAGCTCTTTGTG-3′

[a] F, forward; R, reverse. SpeI, XhoI, SacII, and AscI sites are underlined.

with an immunoglobulin G (IgG)-conjugated column. For conversion of an Fab to a human IgG1, the variable heavy-chain region (V_H) and the variable and constant light-chain region (V_L C_L) of an Fab fragment were amplified by PCR (15 cycles of amplification at 94°C for 2 min, 55°C for 2 min, and 72°C for 2 min) with primers designed for each clone (Table 1). After digestion with SpeI and XhoI for the V_H region and SacII and AscI for the V_L C_L region, the PCR products were subcloned into an IgG1 construction vector. The constructed IgG1 cassette was further cloned into the pCMV-Script expression vector (Fig. 1) (Akahori et al., unpublished). Expression of IgG1 molecules was performed by transfection of the IgG1 expression vectors into CHO-K1 cells by using GenePORTER (Gene Therapy Systems), which allows production and/or secretion of a whole human IgG1 type of Ab in culture fluid.

Enzyme-linked immunosorbent assay (ELISA). To determine the reactivity to or titer of the Abs for strain KU, immunoplates (96-well; Nalgen Nunc International) were coated with highly purified virions (500 ng/well) suspended in PBS(+) for 1 day at 4°C. After a blocking treatment with 5% bovine serum albumin in PBS(+), the plates were washed with PBS(+). For the reactivity study, the plates were then incubated with 50 ng of purified Fab-cp3 in PBS(+) at 4°C overnight. For the titration study, the plates were then incubated with serial dilutions of purified IgG1-formed Abs ranging from 0.01 to 5,000 ng/well in PBS(+) at 4°C overnight. In both cases, after the plates had been washed with PBS(+), a 1:2,500 dilution of peroxidase-conjugated goat anti-human IgG (H+L chain; MBL) was added to each well. One optical density at 492 nm (OD_{492}) unit was defined as the Ab concentration at which the OD_{492} reading was 1.0. To identify virus proteins recognized by the isolated Abs, the immunoplates were coated with 500 ng of purified Fab-cp3 at 4°C overnight, followed by blocking with 5% BSA in PBS(+). Then, unpurified VLPs or recombinant VP4 in the culture supernatant were added. After incubation at 4°C overnight and additional washing with PBS(+), 50 μl of 1:4,500 diluted rabbit anti-HRV antiserum (a mixture of 1:1,500 diluted rabbit anti-KU, anti-AK13, and anti-YO antiserum) was added to each well, followed by incubation at 4°C for 1 day. After the plates had been washed with PBS(+), a 1:2,500 dilution of peroxidase-conjugated goat anti-rabbit IgG (H+L chain; MBL) was added to each well. In all cases, the reactivity of the Abs to Ags was assessed after addition of the substrate.

Assay for virus-neutralizing activity. Screening of crude Fab-cp3 Abs for preliminary selection as to neutralizing activity and determination of the titers of the purified Abs against HRVs or antigenic mutants were determined by the fluorescent focus reduction (FF) method. A total of 25 μl of crude Fab-cp3 or purified Abs in PBS(+) at various concentrations (0.4 to 20 μg/ml) was mixed with an equal volume of virus suspension containing 3.6×10^4 to 14.4×10^4 focus-forming units in Eagle minimum essential medium, followed by reaction at 37°C for 1 h. Aliquots (50 μl) of the mixtures of Abs and viruses were inoculated onto MA104 cells in 96-well culture plates and, after an additional 1 h of incubation at 37°C, 100 μl of fresh Eagle minimum essential medium was added, followed by 16 to 18 h cultivation at 37°C. Fixation in the cold (−80°C), and reaction with the first and second Abs were performed as described previously (49). The neutralization assays were performed in duplicate and at least twice.

Sequence analysis. The nucleotide sequences of V_L and V_H regions were determined with an ABI Prism 320 genetic analyzer by using a BigDye terminator cycle sequencing kit (Applied Biosystems). The T7 primer (5′-TGTAATAC GACTCACTATAG-3′) and the huCH1J primer (5′-ATTAATAAGAGCTAT CCCGG-3′) were used for V_H and V_L sequencing, respectively.

Nucleotide sequence accession numbers. The nucleotide sequence data for the HRV neutralizing Abs reported in the present study have been submitted to the DDBJ database and were assigned the following accession numbers: AB114449 (for 1-2H H chain), AB114450 (2-1D H chain), AB114451 (2-2D H chain), AB114452 (2-3E H chain), AB114453 (2-4F H chain), AB114455 (2-7G H chain), AB114456 (2-9B H chain), AB114457 (2-9D H chain), AB114458 (2-11G H chain), AB114459 (2-12B H chain), AB114460 (3-1G H chain), AB114461 (4-3-C H chain), AB114461 (1-2H L chain), AB114462 (2-1D L chain), AB114463 (2-2D L chain), AB114464 (2-3E L chain), AB114465 (2-4F L chain), AB114466 (2-7G L chain), AB114467 (2-9B L chain), AB114468 (2-9D L chain), AB114469 (2-11G L chain), AB114470 (2-12B L chain), AB114471 (3-1G L chain), and AB114472 (4-3C L chain).

RESULTS

Isolation of Fab forms of Abs with neutralizing activities toward HRVs. After three rounds of panning, the recovered phages were used to infect *E. coli*, which was spread on plates containing ampicillin without infection with helper phages. We picked up 321 colonies and cultured them in 96-well plates. The supernatants, crude Fab-cp3, were directly subjected to analysis of neutralizing activities by means of the FF assay. Among the 321 clones analyzed, 24 appeared to exhibit neutralizing activities toward rotavirus strain KU.

Amino acid sequences of Fab H and L chains. In order to confirm the successful selection of phages with Fab-cp3 specific to strain KU and also to classify the 24 clones, we determined the sequences of variable regions of both their H and L chains. Some redundants were included in the 24 clones (Fig. 2), indicating the specific and successful selection and enrichment of KU-reactive phage Abs. The amino acid sequences of the H chains could be divided into 7 clones and the L chains could be divided into 16 clones (Fig. 2). As a result, 16 of the 24 clones were found to be independent.

Neutralizing activity of the purified Fab fragments. Since the use of crude Fab-cp3 in the FF assay gave ambiguous

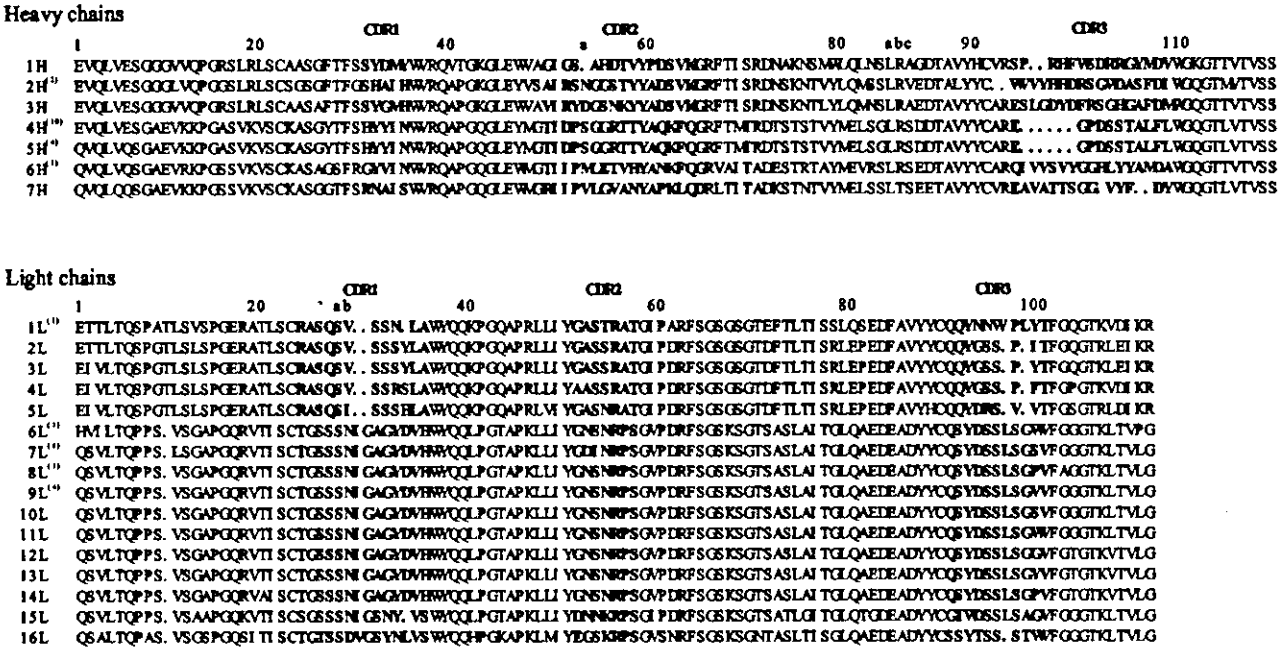

FIG. 2. Amino acid sequences of variable regions of the H and L chains of Abs that appeared to exhibit neutralizing activities toward strain KU. The sequences of CDRs are shown in boldface. Numbers in parentheses indicate number of redundant clones found among the 24 Abs analyzed, with 16 Abs being independent. The combinations of H and L chains in the independent Abs are indicated in Table 2. The numbering of amino acid positions is according to the method of Kabat et al. (23).

results due to contaminants in the *E. coli* culture supernatant, the Fab-cp3 coding phagemid DNAs of the 16 independent clones (2-7G, 3-1G, 2-9D, 2-4F, 2-9B, 1-2H, 4-3C, 2-12B, 2-2D, 2-3E, 2-1D, 2-11G, 1-8A, 2-2G, 2-5G, and 1-4D) were reconstructed to produce Fab-PP fragments (Fig. 1) to facilitate purification. After purification by means of affinity selection, their neutralizing activities toward strain KU were assessed by FF assay. Except for 1-4D, 1-8A, 2-2G, and 2-5G, we could detect the neutralizing activity against strain KU (Table 2). Although the Ag-binding site of each Ab is formed by amino acid residues in the six complementarity-determining regions (CDRs) of the H and L chains, the contribution of CDR3 of the H chain to Ag specificity is greatest among them in a usual case, especially that to protein Ags (21). Judging from this, the two H chains, 4H and 5H, are essentially the same (Fig. 2), and the 12 clones exhibiting the neutralizing activities toward KU could be classified into three groups (Table 2). We finally selected 1-2H, 2-3E, and 2-11G as representative clones of the three group, and further investigations were carried out on these three.

Virus proteins recognized by Abs. The reactivity of the three representative Fab fragments was examined by means of ELISA, their specific reactivity with strain KU being shown (Fig. 3A). Although an isolated VP4 molecule could expose its neutralizing epitope on the surface, VP7 exposed its epitope only when the molecule was embedded in inner proteins VP2 and VP6 (49). Therefore, we prepared two kinds of VLP, VLP-V2/6 and VLP-2/6/7, and recombinant VP4. As shown in Fig. 3B, the targeted virus protein of 1-2H and 2-3E was found to be VP4, and that of 2-11G was found to be VP7. A Western blot analysis to confirm the ELISA results was unsuccessful (data not shown).

Neutralizing activities toward various HRV strains. After conversion of the Fab form into an IgG1 Ab, we analyzed the neutralizing activities of the three Abs against 13 HRVs by means of the FF assay (Table 3). The 1-2H Ab neutralized 7 strains—S2, L26, KU, Wa, YO, Hosokawa, and WI61—all of which exhibited either P[4] or P[8] type specificity on VP4. The 2-3E Ab showed neutralizing activities toward 8 strains—M37,

TABLE 2. Neutralization activity of Fab-PP forms of Abs to strain KU

Clone (n)[a]	H chain	L chain	Antibody concn (μg/ml)[b]
2-7G (1)	4H	6L	1.6
3-1G (1)		7L	8.0
2-9D (4)		9L	1.6
2-4F		10L	1.6
2-9B		12L	1.6
1-2H	5H	13L	1.6
4-3C (1)		8L	1.6
2-12B		11L	1.6
2-2D		14L	1.6
2-3E (1)	2H	1L	1.6
2-1D		15L	1.6
2-11G	1H	2L	1.6
1-8A	3H	5L	>40.0
2-2G	6H	4L	>40.0
2-5G		3L	>40.0
1-4D	7H	16L	>40.0

[a] n = number of redundant clones found among the 24 clones analyzed.
[b] Concentration of antibodies required for a 50% reduction of the fluorescent focus in the FF assay.

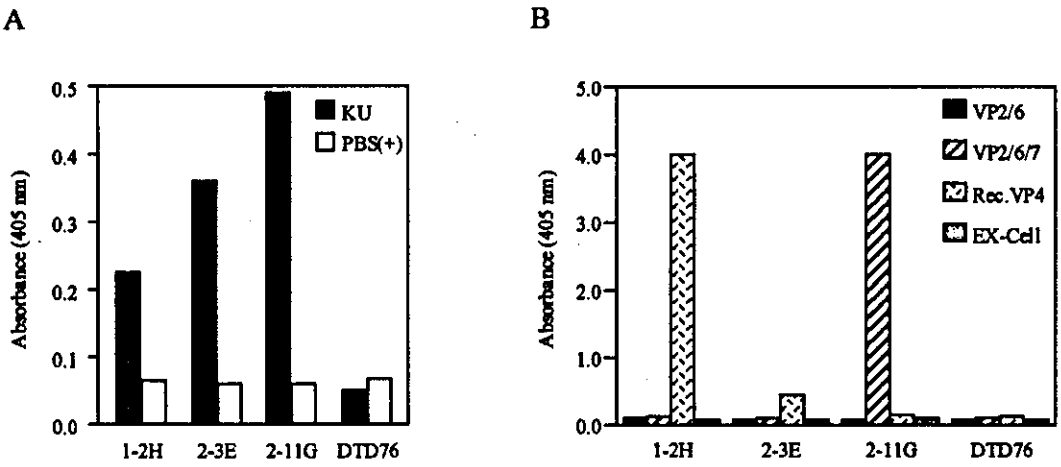

FIG. 3. ELISA of the three Abs (purified Fab-cp3 forms). (A) Reactivities of the three Abs to strain KU. Purified virions suspended in PBS(+) were used as Ags. (B) Virus proteins directed by the three Abs. The culture fluids of Tn5 cells containing VLP-VP2/6, VLP-VP2/6/7, or recombinant VP4 were used as Ags. EX-Cell was the culture medium of Tn5. DTD76 is anti-diphtheria toxin Ab isolated from AIMS4, which was used as a negative control. VP2/6, VLPs constructed with VP2 and VP6; VP2/6/7. VLPs constructed with VP2, VP6, and VP7; Rec.VP4, recombinant VP4.

1076, McN13, KU, Wa, YO, Hosokawa, and WI61—all of which are classified as either a P[6] or P[8] virus strain. The 2-11G Ab reacted with four strains—M37, KU, Wa, and K8—all of which are G1 strains. The conclusion that the 2-11G Ab reacts with VP7 with the G1 serotype was further supported by using two VP7 single gene reassortants, UK/Wa and UK/DS1. The former has VP7 of G1 and VP4 of P[5] specificity, and the latter has VP7 of G2 and VP4 of P[5] specificity. The 2-11G Ab neutralized only the former strain. Of the 13 HRVs examined, 11 were neutralized by any of the three Abs. The concentration of Abs required for 50% neutralization ranged from 0.8 to 20 μg/ml, which corresponds to 5.3 to 133 nM.

Neutralizing activities toward antigenic mutants. We also examined the neutralizing activities of the three representative Abs isolated in the present study with the mouse MAb-resistant mutants prepared in our previous studies (45–47). All of the three Abs of the Fab-cp3 form turned out to neutralize all of the mutants examined (Table 4). The concentration of the each Ab required for 50% reduction of the fluorescent focus in the FF assay was <1.6 μg/ml.

Titers of the Abs to strain KU. We determined the titers of three Abs (IgG1 form) to strain KU by ELISA (Fig. 4). All three Abs showed dose-dependent binding. There was no correlation between the absorbance density and the neutralizing activity of each Ab. The values for one OD_{492} unit for the 1-2H, 2-3E, and 2-11G Abs were 0.70, 0.75, and 1.8 μg/ml, respectively.

DISCUSSION

Ab libraries constructed with a means of a phage-display system are convenient for the rapid isolation of Abs specific for various Ags (2, 27, 31, 44, 52). Recently, several recombinant human Fab fragments exhibiting neutralizing activity toward viruses such as human immunodeficiency virus types 1 and 2, Ebola virus, measles virus, Puumala virus, and respiratory syncytial virus were prepared by means of a phage display system (1, 3, 6, 7, 28, 37, 50). Since the amount of surface proteins with neutralization epitopes on viruses is small and the immunogenicity of inner proteins is quite high, it is generally much more difficult to obtain MAbs with neutralizing capacity than non-neutralizing MAbs. This requires some modifications of the panning and/or screening processes. For example, blockade of a common, nonneutralizing epitope with a representative Fab has been used in panning assays to isolate respiratory syncytial virus-neutralizing human MAbs (50). In the present study, we used highly purified virion for panning and directly screened numerous clones by means of rapid microneutralization FF tests, which have been found to be very efficient for screening neutralizing MAbs with common mouse hybridoma technology (49).

We used HRV strain KU as the Ag for panning, since strain KU exhibits representative G1 and P[8] specificity, which is the

TABLE 3. Neutralization of HRVs and reassortants by purified human IgG1s

Strain	P genotype	G serotype	Antibody concn (μg/ml)[a]		
			1-2H	2-3E	2-11G
S2	P[4]	G2	4–20	>20	>20
L26	P[4]	G12	0.8	>20	>20
M37	P[6]	G1	>20	4	0.8
1076	P[6]	G2	>20	4	>20
McN13	P[6]	G3	>20	4	>20
KU	P[8]	G1	8	1.6	0.4
Wa	P[8]	G1	4	4	4–20
YO	P[8]	G3	8	1.6	>8
Hosokawa	P[8]	G4	4	0.8	>20
WI61	P[8]	G9	4–20	0.8–4	>20
AU-1	P[9]	G3	>20	>20	>20
K8	P[9]	G1	>20	>20	4
69M	P[10]	G8	>20	>20	>20
UK/Wa	P[5]	G1	>20	>20	4
UK/DS-1	P[5]	G2	>20	>20	>20

[a] Antibody concentration that reduced the fluorescent focus count by >50% in the neutralization test (FF assay).

TABLE 4. Neutralization activity of purified antibodies (cp3 form) to antigenic variants

Strain	Mutant protein	No. of virus-infected cells with:[a]											
		1-2H at:			2-3E at:			2-11G at:			DTD76 at:		
		1.6 μg/ml	8 μg/ml	40 μg/ml	1.6 μg/ml	8 μg/ml	40 μg/ml	1.6 μg/ml	8 μg/ml	40 μg/ml	1.6 μg/ml	8 μg/ml	40 μg/ml
V-1E6	VP4	63	69	60	4	2	1	14	2	14	296	378	358
V-1F2	VP4	64	76	43	45	108	21	10	5	1	350	462	368
V-1S3	VP4	31	32	19	127	91	18	26	9	4	312	302	304
V-2C2	VP4	87	46	56	9	3	4	16	0	2	296	342	344
V-4D7	VP4	68	13	12	6	1	0	35	3	4	396	222	354
V-6B11	VP4	21	17	11	8	63	4	3	1	0	212	324	240
V-3C7	VP7	121	123	81	14	17	11	19	3	2	482	532	554
V-4C2	VP7	96	87	120	20	9	5	23	2	3	596	660	656
V-5H1	VP7	146	156	90	13	4	4	46	1	1	372	395	355
V-6A11	VP7	57	84	79	57	23	27	24	8	6	425	554	564
V-KU4	VP7	47	22	14	3	4	0	6	0	3	206	186	206
KU	-	26	15	13	3	3	3	52	3	5	282	300	305

[a] That is, the numbers of virus-infected cells detected by the FF method (see Materials and Methods). Virus-positive cells in a one-ninth area of one well of a 96-well tissue culture plate were counted. Results are presented as the means for two independent experiments performed in duplicate.

most prevalent HRV serotype worldwide (24), and since the neutralization epitopes on VP4 and VP7 of the strain have well been characterized by using mouse MAbs (36, 45, 47, 49). The three human Abs characterized (1-2H, 2-3E, and 2-11G) are specific to P[8], P[4]; P[8], P[6]; and G1 HRVs, respectively. In particular, the former two are broadly reactive with a wide spectrum of HRVs. Since a total of 15 G serotypes have been defined for rotavirus and at least 10 G serotypes have been isolated from humans, it is desirable to prepare such broadly reactive heterotypic Abs for therapeutic purposes. The reactivity of the three Abs covered most HRV strains, and they indeed neutralized 11 of the 13 HRVs examined. In previous studies, an Ab response to cross-reactive neutralization epitopes (YO-2C2 epitopes) was observed much more frequently in schoolchildren and adults than in infants (13, 48). Since the library was constructed from the tissues of adults, who would have been repeatedly infected with HRVs with distinct serotypes and would have immunological memory for cross-reactive neutralization epitopes, cross-reactive Abs may have been readily selected in the present study. In other words, a cross-reactive immune response should be common in the immune system in humans, particularly in adults, infected with rotaviruses.

A number of murine MAbs have been prepared for rotaviruses by means of conventional hybridoma technology. Although many of them were directed to the inner protein VP6,

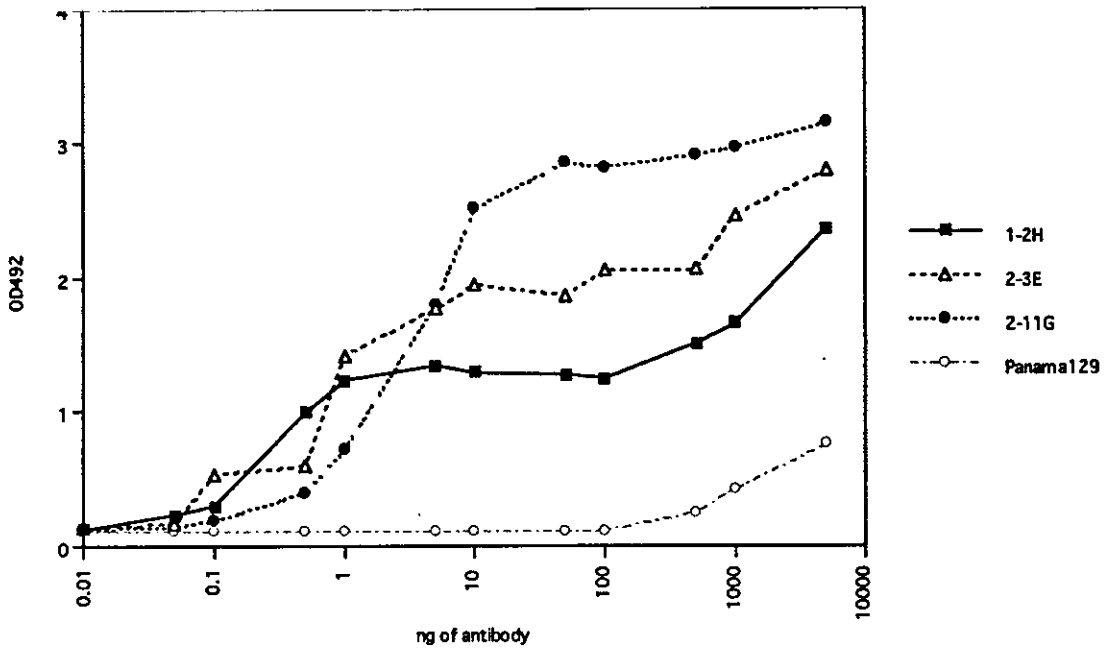

FIG. 4. Abs titration curves on ELISA. The reactivities between purified IgG1-formed Abs and purified virions of strain KU were assessed. The assay was performed in duplicate, and the mean data are plotted. Panama129 is the IgG1-formed anti-influenza virus Ab isolated from the AIMS4 library and converted to the IgG1 form as described in Materials and Methods.

some were directed to VP7 and VP4. However, the number of neutralizing MAbs with hetetrotypic specificity was limited. In particular, ones directed to HRVs were few; i.e., there was only one to VP7 and seven to VP4 (26, 36, 45–47, 49). They have been useful for the analysis of heterotypic neutralization epitopes on VP7 and VP4. By analyzing the mutants resistant to each of the neutralizing mouse MAbs directed to VP4 or VP7 of HRVs, critical amino acids in the neutralization epitopes have been identified (29, 45, 46). It has been shown that the cross-reactive neutralization epitopes on VP4 are the 305th, 385th, 392nd, 428th, 433rd, and 439th amino acid residues (26, 46, 47) and that the G1-specific epitopes on VP7 are the 94th and 96th residues (45). Furthermore, by means of neutralization tests on various combinations of MAb-resistant mutants and MAbs, operational maps of the neutralization epitopes have been constructed (26, 36). We examined the reactivities of the three human Abs isolated in the present study with the MAb-resistant mutants prepared in our previous studies (45–47): mutants resistant to anti-VP4 MAbs (YO-2C2, KU-6B11, YO-1S3, ST-1F2, KU-4D7, and YO-1E6) and mutants resistant to anti-VP7 MAbs (KU-2, KU-4, KU-3C7, KU5H1, KU-6A11, and YO-4C2). These three Abs turned out to neutralize all of the mutants examined (Table 4). This finding strongly suggests that the human Abs isolated in the present study recognize neutralization epitopes distinct from those recognized by mouse MAbs obtained to date. These results could have been predicted, since the specificity showed by the human Abs, such as 1-2H Ab to P[4] and P[8] and 2-3E Ab to P[6] and P[8], had not been shown by mouse MAbs isolated to date. These results imply that the cross-reactive neutralization epitopes recognized by humans, in particular adults, infected with rotaviruses and by mice immunized with rotaviruses are quite distinct. We are now attempting to prepare mutants resistant to each human Ab for analysis of the neutralization epitopes recognized by them.

The mechanism of protective immunity against rotavirus infection has not been well elucidated. Both humoral and cellular immunity are likely to be involved in the protection from rotavirus infection (11, 24, 40). The mucosal Ab response has been believed to be effective for such protection. Furthermore, passive immunity has also been found to be effective (40). Maternal transfer of anti-rotavirus immunoglobulins protects babies from rotavirus infection. Oral administration of bovine immunoglobulins, mouse MAbs, and human immunoglobulins has been found to be effective for protecting suckling mice from rotavirus infection (9, 30, 41). In addition, therapeutic reports on the passive immunity of children with rotavirus diarrhea have also been published. Guarino et al. reported that the oral administration of human serum immunoglobulins to children with rotavirus-induced diarrhea resulted in a faster recovery from the disease (15), even though the children were immunocompromised due to human immunodeficiency virus infection (16). In contrast, there have also been reports showing no clinical effect of oral administration of bovine immunoglobulins for prophylaxis or therapy for HRV infection (4, 10). We are now examining a mouse model to determine whether the human MAbs prepared in the present study are effective and practically relevant to immunotherapy and/or prophylaxis for diseases caused by rotavirus.

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