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Decay-accelerating factor CD55 is identified as the receptor for echovirus 7 using CELICS, a rapid immuno-focal cloning method

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Using an anti-receptor mAb that blocks the attachment of echovirus 7 and related viruses (echoviruses 13, 21, 29 and 33), we have isolated a complementary DNA clone that encodes the human decay-accelerating factor (CD55). Mouse cells transfected with the CD55 clone bind echovirus 7, and this binding is blocked by the anti-receptor mAb. The method used (CELICS) allows rapid and direct cloning of genes encoding cell surface receptors. It is based on episomal replication and high efficiency expression of complementary DNA clones in the vector pCDM8 in COS or WOP cells, in conjunction with a sensitive immuno-focal screen that uses antibody probes linked to β-galactosidase. Receptor positive cells were identified by a colour change and isolated individually using a micromanipulator. DNA extracted from a small number of cells was then cloned directly in *Escherichia coli*.

Key words: CD55/CELICS/cloning method/DAF/virus receptor

Introduction

Cell surface molecules that act as virus receptors are important primary determinants of virus cell tropisms and host range. To date, a number of virus receptors have been identified and the genes encoding some of these have been cloned by screening complementary DNA (cDNA) and/or genomic DNA libraries with anti-receptor antibodies. However, cloning receptor genes is technically demanding and labour intensive. Established cloning procedures have included: (i) screening pools of transformed cells for the presence of the receptor, with subdivisions of pools after each round of screening (Wong *et al.*, 1985; Sims *et al.*, 1988; D'Andrea *et al.*, 1989; Naglich *et al.*, 1992); (ii) 'panning' (Aruffo and Seed, 1987; Seed and Aruffo, 1987; Simmons *et al.*, 1988; Fawcett *et al.*, 1992); (iii) cloning by fluorescence-activated cell sorting (FACS; Yamasaki *et al.*, 1988); and (iv) the introduction of selectable genes using a retroviral transduction system (Albritton *et al.*, 1989; O'Hara *et al.*, 1990). Cloning by 'panning' and FACS involves three to four rounds of selection. Moreover, these methods are based on enrichment rather than focal identification of clones; thus it is not known whether the desired clone is present until several rounds of selection and analysis have been carried out.

To obviate the need for repeated rounds of screening and/or amplification of enriched cell populations, we have developed a strategy for cloning genes directly from cDNA libraries, which we have called CELICS (cloning by enzyme-linked immuno-colour screening). CELICS is based on episomal replication and high efficiency expression of cDNA clones in the vector pCDM8 and in COS and WOP cells (Aruffo and Seed, 1987; Seed and Aruffo, 1987), in conjunction with a modified version of a sensitive immuno-focal assay (Clapham *et al.*, 1992) that identifies antigen-positive cells by virtue of them binding a β-galactosidase (β-gal) antibody conjugate which leads to a blue colouration in the presence of the chromogenic indicator 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-gal). Single blue 'receptor-positive' cells are then isolated free of other cDNA transformants using a micromanipulator, and their plasmid DNA extracted (Hirt, 1967) and cloned directly into *Escherichia coli* using a high efficiency electroporation protocol (Dower *et al.*, 1988).

In this report we describe the cloning and identification of the complement regulatory protein, decay-accelerating factor (CD55), as the receptor for echovirus 7 and related viruses using CELICS.

Results

To establish the CELICS method we first determined that cells expressing the poliovirus receptor (PVR) could be stained blue using anti-PVR antibody mAb 280 (Minor *et al.*, 1984), an anti-mouse IgG antibody conjugated to β-galactosidase and X-gal. This was shown by transforming WOP and COS cells with a PVR cDNA clone in the high expression vector pCDM8 (Aruffo and Seed, 1987; Seed and Aruffo, 1987). PVR has been cloned and identified as a cell surface glycoprotein and is a member of the immunoglobulin superfamily whose function is unknown (Mendelsohn *et al.*, 1989). At 48 h post-transfection, the cells were treated with the antibodies plus X-gal; blue staining of the cells was readily observed in a manner responsive to the amounts of cDNA used. For example, when ~10 μg of the PVR clone DNA were transfected into 10^7 cells, ~10% of the cells stained blue. Using 50 μg of DNA, ~50% of cells stained blue. This result was obtained for COS cells as well as WOP cells, even though COS cells are naturally PVR-positive. No blue cells were observed when control non-transformed COS cells were stained, suggesting that detection of PVR by this method requires a high level of expression such as that provided by pCDM8.

We then investigated whether the method was sensitive enough to detect rare cDNA clones. We therefore trans-

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Table I. Detection level of the CELICS screen

PVR clone dilution in HepG2 library	10^3	10^4	10^5	10^6
Number of blue cells in 10^7 screened	1465	129	13	2

fected COS cells using a HepG2 cDNA library that had been 'spiked' with known amounts of the PVR cDNA clone and stained 48 h later. As shown in Table I, blue cells were detectable when the PVR clone was diluted at a ratio of $1:10^6$ with the library, and no blue cells were observed in the 'non-spiked' sample (the HepG2 library used in this experiment was confirmed as being PVR-negative using PCR; data not shown). Typically, cDNA libraries contain ~10^6 primary clones. These results therefore suggest that the CELICS screening method is sensitive enough to detect rare receptor cDNA clones.

Having established the sensitivity of the immuno-focal screening method, the next step was to determine whether receptor cDNA clones could be rescued from a few isolated blue 'receptor-positive' cells. We therefore repeated the transfection described above using the library 'spiked' with the PVR clone at $1:10^4$; at 48 h, cell monolayers were gently resuspended as single cells and stained. Many (~150) blue cells were observed and 10 of these were isolated using a micromanipulator (Figure 1).

Episomal DNA was then extracted from the 10 blue cells (Hirt, 1967); one fifth of this DNA was used to transform *E.coli* (Dower *et al.*, 1988). Of the 40 *E.coli* colonies which resulted, three were shown to contain the PVR clone, as confirmed by restriction enzyme digestion of small-scale preparations of their plasmid DNA (Birnboim and Doly, 1979) and by transfection of this DNA into COS cells followed by antibody staining using mAb 280 (Clapham *et al.*, 1992).

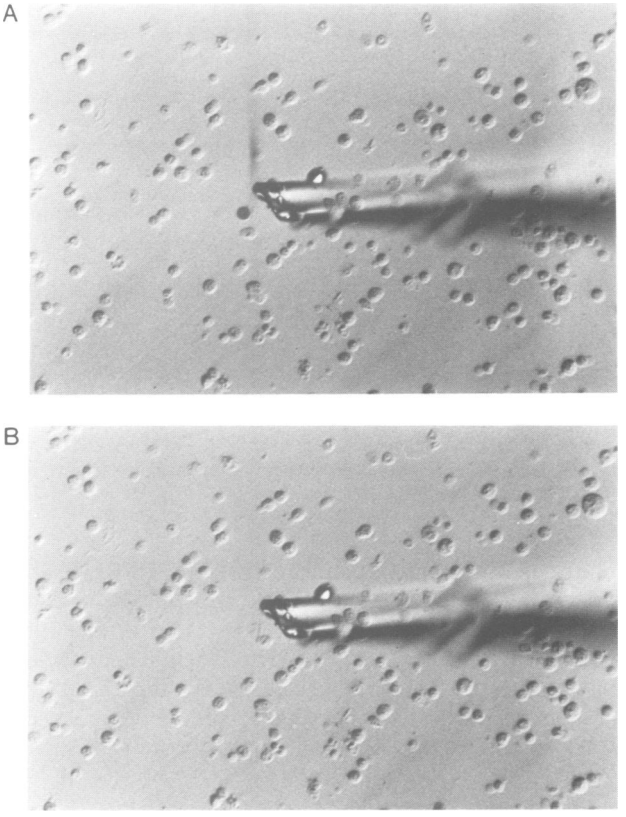

Fig. 1. A photomicrograph of a blue PVR cDNA-positive COS cell (**A**), being isolated using a Narishige micromanipulator (**B**). COS cells were transfected with a HepG2 cDNA library in pCDM8 that had been 'spiked' with a PVR cDNA clone in the same vector at a ratio of $1:10^4$. After 48 h expression, cell monolayers were resuspended and PVR-positive cells were identified using an immuno-focal screen (Clapham *et al.*, 1992) with an anti-PVR antibody mAb 280 (Minor *et al.*, 1984).

Cloning of intercellular adhesion molecule-1 (ICAM-1) using CELICS

Having established that cDNA clones could be recovered from only a few receptor-positive cells, we then determined whether CELICS could be used to isolate primary cDNA clones. A cytokine-stimulated human umbilical vein endothelial cell (HUVEC) cDNA library in pCDM8 was transfected into COS cells (Chu *et al.*, 1987); after 48 h expression the cells were screened using an antibody (mAb 11C8) against the cellular receptor for the major group of human rhinoviruses, ICAM-1 (Greve *et al.*, 1989). Approximately 10^8 cells were examined; nine blue cells were found, of which three were isolated using a micromanipulator. No blue cells were found in antibody-treated control COS cells. One fifth of the episomal DNA extracted from the three blue cells was used to transform *E.coli*; 228 colonies were obtained. A cDNA clone encoding ICAM-1 was identified by screening 20 of these by restriction enzyme digestion analysis of small-scale preparations of their plasmid DNA (Birnboim and Doly, 1979) and by transfection into COS cells followed by antibody staining using mAb 11C8 (Clapham *et al.*, 1992).

Cloning of CD55

Having established the method, CELICS was then used to clone the gene encoding the receptor for echovirus 7 and related viruses. We used an anti-echovirus receptor antibody, mAb 854, produced as described previously (Minor *et al.*, 1984), which was shown to block the binding of echoviruses 7, 13, 21, 29 and 33 to RD cells, but not echovirus 1 or 8, or poliovirus (unpublished data). WOP cells transfected with the HUVEC cDNA library were screened with mAb 854; in the first round seven blue 'receptor-positive' cells were isolated. The episomal DNA extracted from these cells was used to transform *E.coli* (Dower *et al.*, 1988) and 348 colonies were obtained. However, because WOP cells are smaller and less easy to isolate cleanly than COS cells, the *E.coli* colonies were pooled and a small-scale preparation of their plasmid DNA (Birnboim and Doly, 1979) was used in a second round of WOP cell transfection and staining. In the second round, many more blue cells were observed; ~50 were isolated and aspirated into fresh PBS containing 0.5% bovine serum albumin (BSA) in a 35 mm Petri dish for re-picking to remove contaminating white cells. In all, 14 blue cells were re-isolated and their episomal DNA extracted and transformed into *E.coli*, as described above. One fifth of this was used to transform *E.coli*; of the 332 colonies which resulted, 12 were examined by transfection of small-scale preparations of their plasmid DNA (Birnboim and Doly, 1979) into WOP cells. Three were found to convey mAb 854 binding, as determined by antibody staining (Clapham *et al.*, 1992), and to contain a cDNA insert of 1.5 kb. The partial DNA sequence of

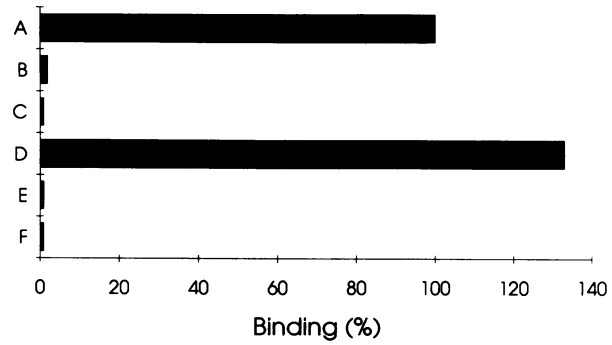

Fig. 2. Binding of ^{35}S-radiolabelled echovirus 7 (Wallace strain) to cells. (**A**) RD cells; (**B**) RD cells pre-incubated with mAb 854; (**C**) WOP cells; (**D**) WOP cells expressing CD55; (**E**) WOP cells expressing CD55 pre-incubated with mAb 854; and (**F**) WOP cells expressing ICAM-1. Results are shown as the percentage relative to [^{35}S]echovirus 7 binding to RD cells.

one of these clones was compared with the EMBL database and a perfect match was found with cDNA encoding CD55, or decay-accelerating factor (Caras *et al.*, 1987). All three clones were identical by restriction endonuclease mapping; one was extensively mapped (using 35 enzymes) and shown to be identical to the published sequence of CD55 (Caras *et al.*, 1987).

CD55 is the receptor for echovirus 7

To demonstrate that CD55 is the receptor for echovirus 7, the binding of purified ^{35}S-radiolabelled echovirus 7 to CD55 cDNA-transfected WOP cells was investigated. WOP cells transfected with the CD55 cDNA clone bound [^{35}S]echovirus 7 at a level similar to permissive rhabdomyosarcoma (RD) cells, whereas the control ICAM-1-expressing WOP cells did not. mAb 854 reduced echovirus 7 binding to background levels (Figure 2).

These results demonstrate that echovirus 7 binds to CD55 and, since mAb 854 blocks echovirus 7, 13, 21, 29 and 33 infection of RD cells, strongly suggest that CD55 is the receptor for this group of echoviruses. It should be noted, however, that the expression of CD55 on WOP cells did not confer permissiveness for echovirus 7 replication. Repeated attempts to infect WOP and Chinese hamster ovary (CHO) cells expressing CD55 were unsuccessful.

Discussion

Strategies for cloning eukaryotic cell surface receptors, such as 'panning' and FACS, require multiple rounds of selective enrichment of and screening for the desired cDNA clone. To obviate the need for multiple rounds we chose to use a direct cloning strategy based on immunocolour screening and micromanipulation, which we have called CELICS. Using this technique and an anti-receptor antibody (mAb 854) we have cloned the echovirus 7 receptor (CD55), the decay-accelerating factor. CELICS provides a relatively simple approach for cloning virus receptors, or indeed any cell surface protein against which mAbs are available. The cloning of an ICAM-1 cDNA clone in one round using CELICS exemplifies the approach. ICAM-1 cDNA was originally cloned by 'panning', and three rounds of enrichment were required (Simmons *et al.*, 1988). Thus, by comparison, CELICS is quicker and more efficient. Moreover, direct examination of cDNA libraries by CELICS makes it possible to avoid the frustration of repeatedly attempting to enrich for clones from libraries that are in fact negative for the receptor cDNA of interest.

Despite the fact that COS cells naturally express PVR, CELICS was used to isolate a PVR cDNA clone from COS cells that had been transfected with a PVR 'spiked' library. This indicates that it is not essential that the target cells used are receptor-negative, so long as the native level of expression is sufficiently low to allow differential staining of transformed and non-transformed cells. This is a further advantage over other methods. For example, when cloning by 'panning' it is essential that the target cells are receptor-negative prior to transfection with cDNA libraries. However, it should be noted that we could not use COS cells for the cloning of CD55 because background expression was too high. A further advantage is that CELICS allows the status of a cDNA library to be established quickly. CELICS can also be used to clone genes encoding internal proteins. In this respect it is very similar to the method of Horst *et al.* (1991) in which immunoperoxidase was used to stain internal proteins of transformed cells.

The echoviruses (32 serotypes) are members of the enterovirus genus of the picornaviridae family and are associated with a wide spectrum of diseases, ranging from rashes to meningitis (Kibrick, 1964; Melnick, 1990). The receptor for echoviruses 1 and 8 has been identified as the integrin VLA-2 (Bergelson *et al.*, 1992), but other echoviruses have been shown to use different receptor(s) (Bergelson *et al.*, 1993). The anti-echovirus receptor antibody used in this report (mAb 854) blocks the binding of echoviruses 7 and other serotypes to RD cells, but has no effect on the binding of echoviruses 1 and 8, or poliovirus (unpublished data). In this study we have shown that echovirus 7 uses the glycosyl phosphatidylinositol (GPI)-anchored protein CD55 or decay-accelerating factor (DAF) as a receptor. This is the first report of a naturally occurring GPI-anchored protein functioning as a virus receptor. A GPI-anchored CD4/Thy-1 chimeric molecule has been shown previously to serve as a functional receptor for HIV (Jasin *et al.*, 1991).

CD55 is a protein of M_r ~70 kDa whose function is to regulate complement activity (Lublin and Atkinson, 1989). It both dissociates (decay-accelerates) and prevents the assembly of C3/C5 convertases in both the classic and alternative pathways of the complement system (Lublin and Atkinson, 1989). CD55 is composed of four contiguous repeats of ~60 amino acids that share sequence similarity with other complement binding proteins such as membrane cofactor protein (CD46), the interleukin-2 receptor and a subunit of clotting factor XIII (Lublin and Atkinson, 1989). Two other proteins that regulate complement activity have been shown to act as virus receptors; membrane cofactor protein CD46 is the receptor for measles virus (Dorig *et al.*, 1993; Naniche *et al.*, 1993), and CR2 is the receptor for Epstein–Barr virus (Fingeroth *et al.*, 1984; Moore *et al.*, 1987; Tanner *et al.*, 1987).

CD55 is expressed in a wide range of tissues and human cell lines in culture (Medof *et al.*, 1987; Lublin and Atkinson, 1989). However, expression does not appear to be sufficient *per se* to confer permissiveness to cells. For

example, many human CD55-expressing cell lines in culture are poorly permissive or non-permissive for echovirus 7 replication (data not shown). Moreover, WOP cells expressing CD55 are non-permissive for echovirus 7 replication, even though they do allow virus binding comparable with the permissive RD cells. This suggests that the tissue tropisms of echoviruses and the diseases associated with them may be determined not only by CD55, but by other cellular factors too. The involvement of cellular factors other than the receptor in determining the host range and cell tropisms of viruses has been observed. For example, CD4 is the receptor for HIV-1 and yet this molecule alone does not confer permissiveness to mouse cells (Maddon *et al.*, 1986). Similarly, ICAM-1 does not confer permissiveness for rhinovirus 14 to mouse cells. Furthermore, CD44 in conjunction with PVR is a determinant of cell tropism of poliovirus (Shepley and Racaniello, 1994).

The echovirus serotypes blocked by mAb 854 are all haemagglutinating enteroviruses that react with erythrocytes from humans but not from other species (Philipson *et al.*, 1964). CD55 is expressed on human erythrocytes (Medof *et al.*, 1987) and is thus likely to be the protein responsible for echovirus-induced haemagglutination.

In conclusion, we have identified the echovirus 7 receptor as being the complement regulator CD55. The CELICS technique we have used allows rapid and efficient cloning of genes encoding cell surface proteins such as virus receptors; it thus provides a favourable alternative to cloning by 'panning' or FACS. We are presently attempting to clone DNA recovered from individual cells which have been infected by a picornavirus and then permeablized and visualized using anti-viral antibodies.

Materials and methods

Antibodies

The anti-mouse IgG β-gal antibody conjugate was purchased from Amersham (UK) and was used at a dilution of 1/100. The anti-ICAM-1 mAb (mAb 11C8) was a gift from David Simmons (ICRF, Oxford, UK) and was used at 2 μg/ml. The production of the anti-poliovirus receptor antibody (mAb 280) has been described previously (Minor *et al.*, 1984). mAb 280 ascites fluid was used at a dilution of 1/1000. The anti-echovirus receptor antibody (mAb 854) was produced concomitantly with mAb 280 (Minor *et al.*, 1984). mAb 854 was purified by protein A affinity chromatography and was used at 3 μg/ml. All antibodies were diluted with PBS/BSA containing 0.02% sodium azide. Prior to use, diluted antibodies were filtered through a 2 μm membrane (Sartorius, Göttingen, Germany).

DNA transfection of COS and WOP cells

Mouse WOP cells were obtained from Ian Campbell (ICRF, London, UK). COS-7 cells were obtained from the American Type Culture Collection. Both WOP and COS cells were cultured in Dulbecco's modified eagle's medium supplemented with 10% fetal calf serum. The cDNA libraries and the ICAM-1 clone in the high expression vector pCDM8 (Aruffo and Seed, 1987; Seed and Aruffo, 1987) were gifts from David Simmons (ICRF, Oxford, UK). The PVR cDNA used in this study was obtained by PCR amplification of an HT1080 fibroblast cDNA library with PVR cDNA-specific primers, and then cloned into the high expression vector pCDM8 and shown to be functional (unpublished data).

In the studies that determined the detection level of CELICS, 10^7 COS cells were transfected with 10 μg of a HepG2 cell cDNA library that had been 'spiked' with the PVR cDNA clone at various levels. For receptor cloning, 10^8 COS or WOP cells were transfected with 100 μg of a HUVEC cDNA library. Cells were transfected by electroporation (Chu *et al.*, 1987) and seeded at ~10^7 cells per 75 cm^2 flask, and cultured at 37°C in a humidified 5% CO_2 incubator for 48 h to allow expression.

Immuno-focal screening

This method is a modified version of a sensitive immuno-focal assay (Clapham *et al.*, 1992). After expression, cell monolayers were washed twice with 10 ml volumes of PBS. For receptor cloning, cells were suspended in 8 ml PBS/BSA after being incubated for 15 min with 2 ml PBS containing 0.5 mM EDTA. After centrifugation at 250 *g* for 5 min, the cells were resuspended and incubated in the presence of 1 ml anti-receptor antibody for 1 h on ice. After washing three times with 5 ml volumes of PBS/BSA, the cells were resuspended and incubated with 1 ml anti-mouse IgG β-gal antibody for 1 h on ice, washed again three times with PBS/BSA and then resuspended in 10 ml PBS containing 0.05% X-gal, 3 mM $K_4Fe(CN)_6$, 3 mM $K_3Fe(CN)_6$, 1 mM $MgCl_2$ and 0.1% BSA. Cells were then transferred to 90 mm Petri dishes (pre-treated with PBS/BSA; ~10^7 cells/dish) and incubated at room temperature for 2 h. The Petri dishes were then examined for the presence of blue cells, using an inverted light microscope (Nikon, Japan) with the aperture fully open to reduce light scatter. Blue 'receptor-positive' cells were picked individually using a fine capillary tube (pre-treated with PBS/BSA) attached to a micromanipulator (Narishige, Japan) and linked to a syringe (100 μl, Hamilton) via a silicon tube filled with mineral oil. The blue cells were then pooled and their episomal DNA extracted (Hirt, 1967) and used to transform *E.coli* MC1061/P3s (Dower *et al.*, 1988). Transformed *E.coli* were selected for by plating out on nutrient agar containing ampicillin (50 μg/ml) and tetracyclin (10 μg/ml). The *E.coli* colonies generated were then screened by using small-scale preparations of their plasmid DNA (Birnboim and Doly, 1979) in a further round of immuno-focal screening (Clapham *et al.*, 1992). Those cDNA clones able to produce a blue colour in this assay were studied further.

Echovirus 7 binding

10^7 mouse WOP cells were transfected by electroporation with 20 μg of the CD55 cDNA clone in pCDM8 (Aruffo and Seed, 1987; Seed and Aruffo, 1987). Control WOP cells were transfected with no DNA or with 20 μg of an ICAM-1 cDNA clone in pCDM8. Cells were then allowed to express for 72 h by incubation at 37°C in a humidified 5% CO_2 incubator. RD cells and the WOP cell transfectants were then suspended in PBS/EDTA, aliquoted at 10^6 cells per 1.5 ml microcentrifuge tube, and incubated on ice with or without 100 μl mAb 854 (10 μg) for 1 h. Cells were then washed three times with 1 ml volumes of PBS after centrifugation at 250 *g* for 5 min. ^{35}S-radiolabelled echovirus 7 (Wallace stain; 10^5 c.p.m./10^7 $TCID_{50}$) prepared from infected RD cells (Minor *et al.*, 1980) was then added, and the cells incubated for 1 h on ice. Unbound virus was then removed by four washes with PBS and centrifugation, and the bound fraction was quantified by use of a scintillation counter.

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References

Albritton,L.M., Tseng,L., Scadden,D. and Cunningham,J.M. (1989) *Cell*, **57**, 659–666.

Aruffo,A. and Seed,B. (1987) *Proc. Natl Acad. Sci. USA*, **84**, 8573–8577.

Bergelson,J.M., Shepley,M.P., Chan,B.M.C., Hemler,M.E. and Finberg,R.W. (1992) *Science*, **255**, 1718–1720.

Bergelson,J.M., St John,N., Kawaguchi,S., Chan,M., Stubdal,H., Moldin,J. and Finberg,R.W. (1993) *J. Virol.*, **67**, 6847–6852.

Birnboim,H.C. and Doly,J. (1979) *Nucleic Acids Res.*, **7**, 1513–1523.

Caras,I.W., Davitz,M.A., Rhee,L., Weddell,G., Martin,D.W. and Nussenzweig,V. (1987) *Nature*, **325**, 545–549.

Chu,G., Hayakawa,H. and Berg,P. (1987) *Nucleic Acids Res.*, **15**, 1311–1326.

Clapham,P.R., McKnight,A. and Weiss,R.A. (1992) *J. Virol.*, **66**, 3531–3537.

D'Andrea,A.D., Lodish,H.F. and Wong,G.G. (1989) *Cell*, **57**, 277–285.

Dorig,R.E., Marcel,A., Chopra,A. and Richardson,C.D. (1993) *Cell*, **75**, 295–305.

Dower,W.J., Miller,J.F. and Ragsdale,C.W. (1988) *Nucleic Acids Res.*, **16**, 6127–6145.

Fawcett,J., Holness,C.L.L., Needham,L.A., Turley,H., Gatter,K.C., Mason,D.Y. and Simmons,D.L. (1992) *Nature*, **360**, 481–484.
Fingeroth,J.D., Weis,J.J., Tedder,T.F., Strominger,J.L., Biro,P.A. and Fearon,D.T. (1984) *Proc. Natl Acad. Sci. USA*, **81**, 4510–4514.
Greve,J.M., Davis,G., Meyer,A.M., Forte,C.P., Yost,S.C., Marlor,C.W., Kamarck,M.E. and McClelland,A. (1989) *Cell*, **56**, 839–847.
Hirt,B. (1967) *J. Mol. Biol.*, **26**, 365–369.
Horst,E., Wijngaard,P.L.J., Metzelaar,M., Bast,E.J.E.G. and Clevers,H.C. (1991) *Nucleic Acids Res.*, **19**, 4556.
Jasin,M., Page,K.A. and Littman,D.R. (1991) *J. Virol.*, **65**, 440–444.
Kibrick,S. (1964) *Prog. Med. Virol.*, **6**, 27–70.
Lublin,D.M. and Atkinson,J.P. (1989) *Annu. Rev. Immunol.*, **7**, 35–58.
Maddon,P.J., Dalgleish,A.G., McDougal,J.S., Clapham,P.R., Weiss,R.A. and Axel,R. (1986) *Cell*, **47**, 333–348.
Medof,M.E., Walter,E.I., Rutgers,J.L., Knowles,D.M. and Nussenzweig,V. (1987) *J. Exp. Med.*, **165**, 848–864.
Melnick,J.L. (1990) In Fields,B.N. and Knipe,D.M. (eds), *Virology*. Raven Press, New York, Vol. I, pp. 549–605.
Mendelsohn,C.L., Wimmer,E. and Racaniello,V.R. (1989) *Cell*, **56**, 855–865.
Minor,P.D., Schild,G.C., Wood,J.W. and Dandawate,C.N. (1980) *J. Gen. Virol.*, **51**, 147–156.
Minor,P.D., Pipkin,P.A., Hockley,D., Schild,G.C. and Almond,J.W. (1984) *Virus Res.*, **1**, 203–212.
Moore,M.D., Cooper,N.R., Tack,B.F. and Nemerow,G.R. (1987) *Proc. Natl Acad. Sci. USA*, **84**, 9194–9198.
Naglich,J.G., Metherall,J.E., Russell,D.W. and Eidels,L. (1992) *Cell*, **69**, 1051–1061.
Naniche,D., Varior-Krishnan,G., Cervoni,F., Wild,T.F., Rossi,B., Rabourdin-Combe,C. and Gerlier,D. (1993) *J. Virol.*, **67**, 6025–6032.
O'Hara,B., Johann,S.V., Klinger,H.P., Blair,D.G., Rubinson,H., Dunn,K.J., Sass,P., Vitek,S.M. and Robbins,T. (1990) *Cell. Growth Diff.*, **1**, 119–127.
Philipson,L., Bengtsson,S., Brishammar,S., Svennerholm,L. and Zetterqvist,O. (1964) *Virology*, **22**, 580–590.
Seed,B. and Aruffo,A. (1987) *Proc. Natl Acad. Sci. USA*, **84**, 3365–3369.
Shepley,M.P. and Racaniello,V.R. (1994) *J. Virol.*, **68**, 1301–1308.
Simmons,D., Makgoba,M.W. and Seed,B. (1988) *Nature*, **331**, 624–627.
Sims,J.E. *et al.* (1988) *Science*, **241**, 585–589.
Tanner,J., Weis,J., Fearon,D., Whang,Y. and Kieff,E. (1987) *Cell*, **50**, 203–213.
Wong,G.G. *et al.* (1985) *Science*, **228**, 810–815.
Yamasaki,K., Taga,T., Hirata,Y., Yawata,H., Kawanishi,Y., Seed,B., Taniguchi,T., Hirano,T. and Kishimoto,T. (1988) *Science*, **241**, 825–828.

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