Cloning and Characterization of a Novel Promiscuous Human β-Chemokine Receptor D6*

(Received for publication, July 18, 1997, and in revised form, October 7, 1997)

Robert J. B. Nibbs, Shaeron M. Wylie, Jinying Yang‡, Nathaniel R. Landau‡, and Gerard J. Graham§

From the the Beatson Institute for Cancer Research, Cancer Research Campaign Beatson Laboratories, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, United Kingdom and the §Aaron Diamond AIDS Research Center, Rockefeller University, New York, New York 10016

Members of the chemokine family of chemotactic peptides interact with their target cells through heptahelical cell surface receptors. An understanding of the biochemistry and expression patterns of these receptors is therefore central to our overall understanding of the roles played by chemokines in both physiological and pathological processes. To date, eight receptors for the β-chemokine subfamily have been described. We have recently cloned a novel murine β-chemokine receptor and report here the identification and characterization of a highly homologous human gene termed human D6 (hD6). This is a promiscuous β-chemokine receptor and appears to be able to bind the majority of members of the β-chemokine family. It is, however, specific for this family and shows no detectable affinity for members of the α-chemokine or the C or CXXXC chemokines. Unlike the majority of other chemokine receptors, human D6 does not appear to be able to flux calcium following ligand binding, thus it is currently not clear if this novel receptor is indeed a signaling receptor. Human D6 is expressed in a range of tissues including hemopoietic cells although it appears not to be ubiquitously expressed in hemopoietic cells. Human D6, unlike some other β-chemokine receptors, appears not to be able to function as an entry co-factor for human immunodeficiency virus type 1 (HIV-1) on CD4-expressing cells.

The chemokine family of peptides is defined on the basis of sequence homology and on the presence of variations on a conserved cysteine motif (1, 2). The family can be subdivided on the basis of this motif into two major subfamilies, in which members of each contain four characteristic cysteine residues. This subdivision therefore defines the CC or β-chemokine family in which the first two cysteines are juxtaposed, and the CXC or α-chemokine family in which there is an intervening amino acid between the first two cysteines. Further, two subfamilies have recently been described, the C family, which has only two cysteines in the mature protein (3, 4), and the CXXXC family, which has three intervening amino acids between the first two cysteine residues of the mature protein (5). The sole member of the C family cloned to date is Lymphotactin, and Fractalkine is the only member of the CXXXC family identified thus far.

Chemokines display a range of in vitro and in vivo functions ranging from pro-inflammatory activities on a range of cell types to proliferative regulatory activities. All functions of the chemokine family are believed to be signaled into a responsive cell using members of the G-protein-coupled heptahelical receptor family (6). To date, a number of CC and CXC chemokine receptors have been cloned. In general these receptors are specific for the respective subfamilies, however, they do display complex and overlapping ligand binding profiles. Thus far, four α-chemokine receptors (CXCR1–4) have been described (7–11), and an additional eight β-chemokine receptors are now known (12–21). Other chemokine receptors identified to date include the DARC receptor (22, 23), which recognizes both α- and β-chemokines and a number of virally encoded heptahelical receptors (24–26).

In addition to their likely roles in inflammatory processes, a number of chemokine receptors have recently been implicated as co-factors with CD4 in mediating entry of HIV-1 into CD4 positive cell types. CCR5 appears to be the predominant receptor for mediating entry into monocytes and T cells by M-tropic HIV-1 strains and SIV (27–29), whereas the α-chemokine receptor CXCR4 appears to be a major determinant of entry of T cell-tropic strains of HIV-1 into T cells (10, 11).

We now report the identification and characterization of a novel human β-chemokine receptor that has high structural and functional homology to a murine receptor, which we have recently described (30). This receptor, which we have designated human D6, is promiscuous β-chemokine receptor and appears to bind the majority of members of the β-chemokine family. In contrast to some other CC chemokine receptors, hD6 does not appear to support HIV-1 or SIV entry into CD4-expressing cells. We have, so far, been unable to demonstrate signaling through human D6 following ligand binding, and in keeping with the agreed restrictions on chemokine receptor nomenclature, designation of human D6 as CCR9 awaits such data.

Materials and Methods

Chemokines—All chemokines were purchased from PeproTech (London, United Kingdom) except human HCC-1 and murine JE, which were purchased from R&D systems (Abingdon UK). The murine MIP-1α used in these studies is a nonaggregating variant with wild-type activity as described previously (31). All peptides were maintained at a concentration of 0.1 mg/ml in phosphate-buffered saline.

Cloning of Human D6—Fragments of human D6 were generated by degenerate oligonucleotide-primed PCR using the following primers designed from the murine D6 receptor (30) in two regions that show

* This study was funded in part by grants from the Cancer Research Campaign, the National Institutes of Health (to N. L. R.), and the American Foundation for AIDS Research. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) Y12815.

§ To whom correspondence should be addressed. Tel.: 44-141-330-3982; Fax: 44-141-942-6521; E-mail: GPMA09@udcf.gla.ac.uk.

1 The abbreviations used are: HIV-1, human immunodeficiency virus type 1; SIV, simian immunodeficiency virus; PCR, polymerase chain reaction; CHO, Chinese hamster ovary; RT, reverse transcriptase.
conservation of amino acid sequence between other human and mouse chemokine receptors: at the 5′ end, hD6TCM, 5′-TG(C/T) GTG ATC TTI(T/T) TT(T/T) ATC T(T/T) ACI TG(T/C) ATG-3′; or hD6DKY, 5′-GAC AA(A/G) TA(C/T) CTI GA(A/G) ATC T(T/T) GTI CA(C/T) GC-3′; at the 3′end,hD6TCC, 5′-GTA CAG IAC ICG IGT TACI A/C(G/A) TGT-3′. PCR was performed with variable amounts of MgCl2 (from 1.25 to 2.25 mM), 0.3 μM of each dNTP, and 6 ng/μl of one of the 5′ oligonucleotides and hD6HCC. Genomic DNA template isolated from human lymphocytes was used at 1 μg per reaction. Reactions were incubated for 94 °C for 1 min, 50–52°C for 1 min, and 72 °C for 2 min. Products of expected size were cloned into pCRScript (Stratagene, La Jolla, CA) and sequenced. Two different products were cloned: hD6, and a fragment identical to the previously cloned orphan heptahedral receptor, RDC1. 5′ and 3′ rapid amplification of the hD6 cDNA ends (RACE) was performed using RACE kits from Life Technologies, Ltd. (Paisley, UK). Using oligonucleotide primers designed from the sequenced fragment. Reverse transcribed U937 cDNA was used as a template for the RACE reactions, which had previously been shown to express hD6. Once the start and stop codons were identified, the full-length gene was amplified with Pfu polymerase (Stratagene) in three separate reactions from human lymphocyte DNA. These products were cloned into pCRScript and fully sequenced. The three products were identical.

Generation of CHO and human osteosarcoma Cells Stably Expressing hD6—CHO cells were maintained in special liquid medium (Life Technologies, Ltd.) supplemented with 4 mM glutamine and 10% fetal calf serum. These products were cloned into pCRScript with reverse transcriptase (RT) and one without. 1.2 μg was performed using the RNA PCR core kit (Perkin-Elmer). For each reaction, aliquots were used to amplify actin transcription, ethanol precipitation and 70% ethanol wash. Reactions with hD6 antisense 5′-CGT TCA GCC TCA GCA CTA C-3′ and hD6 antisense 5′-CTG GAG TGC GTA GTC TAG ATG C-3′ (expected size, 447 base pairs); and actin-sense 5′-TCC ATC ATG AAG TGC GTC-3′ with actin-antisense 5′-TAC TCC TGC TTG CTG ATC CAC-3′ (expected size, 246 base pairs). PCR proceeded for 30 cycles of 94 °C for 1 min, 58 °C for 1 min, 72 °C for 1.5 min.

Receptor Binding Studies—All receptor binding studies were carried out as ligand displacement analyses essentially as described previously (30, 35) with cells being plated at 2 × 105 cells/well in 24-well plates overnight at 37 °C prior to binding analysis. Cells were incubated in azide-containing medium (pH 7.4) with a constant amount of radioiodinated murine MIP-1α and displacement analysis carried out using increasing concentrations of unlabeled MIP-1α as outlined in the relevant results. Data was analyzed by the LIGAND program (36).

Cloning and Sequence Analysis of Human D6—Cloning of a novel human receptor-like gene from genomic DNA. These fragments were subsequently used to design oligonucleotide primers for use in RACE PCR reactions to isolate the 5′ and 3′ sequences of the cDNA using human U937 monocytic RNA as a template, known to be positive for hD6 expression. Pfu polymerase was used to generate a full-length receptor cDNA of 1181 base pairs encompassing an open reading frame of 1152 base pairs (GenBank™ accession number: Y12815). The cDNA encodes a protein of 384 amino acids in length (Fig. 1), displaying the characteristic seven transmembrane-spanning domains (dashed lines) and four conserved cysteine residues (underlined C) involved in maintaining receptor structure. hD6 bears a single putative N-linked glycosylation site at the amino terminus (NSS, underlined) and a number of potential phosphorylation sites at the carboxyl terminus, which is rich in serine and threonine residues. The hD6 cDNA displays 71% identity and 86% similarity to the murine D6 receptor, and in common with this protein, it has an alteration in the highly conserved DRYLAIYVA motif seen in other chemokine receptors changing it to DKYIEIVHA in human and murine D6, introducing an additional charged residue into the site (Fig. 1, bold, italicized, and underlined). These particular changes are not observed in any other G-protein-coupled receptors although there is a certain amount of variance in this motif in nonchemokine receptors. Whereas the extent of homology between human and murine D6 (71% identity) is marginally lower than that observed for the murine and human orthologues of other chemokine receptors (generally around 80%), comparison of Southern blots probed with hD6 with the same blots probed at low stringency with murine D6 suggests that the murine D6 gene hybridizes most strongly to this human gene rather than to other receptors in the human genome, implying that these genes are the closest sequence homologues between these two species (data not shown). hD6 has ~40% identity and ~50% similarity to the eight other known human β-chemokine receptors, which is a consistently lower homology than these receptors show to one another, implying that hD6 is a more divergent member of this family. However, the amino terminus of hD6, a region believed to be important in ligand binding, contains a number of tyrosines and acidic residues that are also seen in the other β-chemokine receptors. One potentially important sequence difference between D6 and the other receptors is the alteration of a highly conserved aspartic acid residue in the second transmembrane domain to an asparagine (Fig. 1, labeled #). This aspartic acid is seen in almost all heptahedral receptors with a few signaling competent virally encoded proteins and DARC being the notable exceptions. When this residue is mutated in hCCR5 or other heptahedral receptors certain intracellular effects, such as Ca2+ flux, are no longer observed, although ligand binding is usually unaffected (37–38). This is likely to significantly alter the signaling properties of D6 (see below). Finally, it is notable that the 3′ sequences of hD6 are identical to our expressed sequence tag derived from a human placental cDNA library, and thus hD6 represents the full-length cDNA corresponding to this expressed sequence tag (GenBank™ accession number R82383).

Analysis of the Expression of hD6 in Human Tissues—Tissue
blots containing representative mRNA samples from various human tissues were probed with full-length hD6. Two transcripts were detected of around 4 and 6 kilobases, and as shown in Fig. 2A, expression of the receptor was seen to be very high in the placenta, lower in the liver, with weak but detectable expression seen in the lung and thyroid. On prolonged exposure of these blots, weak expression is also detectable in a range of other tissue types, particularly small intestine and the mucosal lining of the colon. However, it should be mentioned that the contribution of resident leukocytes to this weak expression cannot be discounted, although we have been unable to detect significant expression of hD6 in peripheral blood leukocytes using Northern blotting (see Fig. 2A and below for a discussion of this point). In contrast to the murine D6 receptor (30), hD6 does not appear to be expressed in the spleen; however, it is possible that this could be a reflection of the relatively different cellular composition of the murine and human spleens.

Surprisingly, it has proved difficult to detect expression of hD6 in hemopoietic cells using Northern blot analysis of samples from peripheral blood leukocytes, bone marrow (see Fig. 2A), and the cell lines HL-60, Raji, K562, and THP-1 (data not shown). However, PCR has demonstrated hD6 expression in

Cloning of Human D6

hD6 mRNA was used to clone the hD6 receptor. Putative transmembrane regions are underlined with a dashed line, the four conserved cysteine residues are indicated with an underlined C underneath the sequences, and the hD6 variant of DRYLAIV is in bold type, underlined and italicized. The single putative N-linked glycosylation site is underlined and in bold type. The highly conserved aspartic acid residue, changed to asparagine in hD6 and mD6, is shown with a # symbol above it. The hD6 nucleotide sequence is available from EMBL/GenBank/DBJ under accession number Y12815.

Fig. 1. Protein sequence of hD6, mD6 and eight other β-chemokine receptors. Putative transmembrane regions are underlined with a dashed line, the four conserved cysteine residues are indicated with an underlined C underneath the sequences, and the hD6 variant of DRYLAIV is in bold type, underlined and italicized. The single putative N-linked glycosylation site is underlined and in bold type. The highly conserved aspartic acid residue, changed to asparagine in hD6 and mD6, is shown with a # symbol above it. The hD6 nucleotide sequence is available from EMBL/GenBank/DBJ under accession number Y12815.
RNA prepared from leukocytes derived from umbilical cord blood and in the primitive erythromyeloid leukemic cells lines, K562, and THP-1 monocytic cells. Raji and HL-60 cells remain negative in this more sensitive assay (Fig. 2B). The expression of hD6 in K562 cells correlates well with the documented expression of the murine counterpart of hD6 in primitive bone marrow cells (30).

The general weak hemopoietic expression of this gene suggests that the solid organ expression of hD6 may be due to nonhemopoietic cells or to the activation status and type of resident leukocytes. In situ hybridization studies should distinguish between these two possibilities.

Ligand Binding Profiles of hD6—Ligand displacement analysis was performed on CHO cells stably expressing hD6, and the data from these studies are outlined in Fig. 3. A and B and summarized in Table I. The data indicate that hD6 is a highly promiscuous β-chemokine receptor that displays relatively high affinity binding for the majority of members of the β-chemokine family. The highest affinity ligands for hD6 appear to include murine MIP-1α and MIP-1β and murine JE and human MCP2. Human MCP1 has approximately a 30-fold lower affinity for hD6 than the presumed murine homologue (JE), a similar difference in affinity to that seen with the mouse D6 receptor, which again binds human MCP1 with 30-fold lower affinity than murine JE (30). More recently, it has been suggested that murine MCP5 is the closer murine homologue of MCP1 than JE (39) and indeed this is borne out by our binding analysis that shows murine MCP5 and human MCP1 to have very similar dissociation constants for hD6. Binding to hD6 is also seen with the human chemokines RANTES, MCP3, MCP4, and HCC1, and binding of both murine and human eotaxin is detectable, although this is a relatively lower affinity interaction than that seen with many of the other β-chemokines, and the physiological relevance of this interaction remains to be determined. No binding of the β-chemokine C10 is detectable, and in addition, 1309 appears not to be a ligand for hD6. The specificity of hD6 for the β-chemokines is confirmed by the inability of various α-chemokines (IL8, GroY, IP10, and MIP2) and lymphotactin to displace 125I-labeled mMIP-1α from the receptor. It is noteworthy that in our hands K562 cells display a receptor that exhibits similar properties with respect to affinity and specificity as does hD6 (not shown).

Human and murine D6 exhibit many similarities with respect to ligand binding, and it appears from our recent studies using β-chemokines tested here that were not commercially available at the time of publication of the murine receptor (e.g. MIP4 and 5) that murine D6 displays a similar ligand promiscuity to that seen with hD6 (data not shown). Nevertheless, a number of discrepancies do exist between D6 receptors from these two species. For instance, human MCP2 is a high affinity ligand for hD6 but does not bind to murine D6 (30). However, it remains possible that the murine homologue of MCP2 may exhibit an affinity for murine D6. Also, surprisingly, human MIP-1α has markedly reduced affinity for hD6 compared with murine MIP-1α for mD6; hMIP-1α may not be able to bind to hD6 at physiological concentrations. However, recent results from our laboratory strongly suggest that the human and murine MIP-1α molecules used in this study cannot be considered to be functionally homologous. Thus, we suggest that despite these anomalies it is highly likely that human and murine D6 are functionally analogous.

The basis for the remarkable promiscuity of hD6 is not immediately obvious. It is likely that this is a reflection of the usage of elements common to the majority of β-chemokines in binding such as the clusters of highly conserved residues between cysteines 3 and 4. This assertion is further substantiated by the observations that alterations to the cluster of basic residues in this area substantially affect binding. Thus, weakly binding chemokines such as eotaxin and MCP1 have nonconservative alterations in this area. In addition, the nonproteoglycan binding variant of murine MIP-1α, which we have previously generated by neutralization of two of the basic residues in this cluster (32), shows a 30-fold reduction in affinity for hD6 compared with the wild-type mMIP-1α protein (data not shown). It is important to highlight however that while this region may well be of importance in hD6 binding, it is not a singular requirement as this site is conserved in human MIP-1α, which shows only limited binding to hD6. Therefore it is likely that other regions of the ligands are also important in hD6 binding.

The relatively unique nature of the amino terminus of murine and human D6 suggests that this region may be involved to some extent in defining the ligand promiscuity of this receptor. We are in the process of generating receptor chimeras to test this hypothesis.

We have been interested in determining the signaling competence of hD6 in response to the various binding β-chemokines, however we have been unable to elicit any detectable calcium flux in hD6 expressing HEK293, human osteosarcoma (see below), or CHO cells. This is in contrast to the murine D6

2 Robert J. B. Nibbs, Shaeron M. Wylie, Jinying Yang, Nathaniel R. Landau, and Gerard J. Graham, manuscript in preparation.
receptor and to human CCR1 and CCR5, which in our hands do
signal in response to ligand binding in 293 cells, and may be
indicative of alternative G-protein coupling by the hD6. The
divergent nature of the hD6 carboxyl terminus, the altered
DRYLAIVHA motif, and the absence of the aspartic acid resi-
due in the second transmembrane domain in hD6 (described
above) may conspire with other sequence peculiarities of hD6 to
explain this phenomenon. This matter is currently under in-
vestigation in our laboratory.

It is unclear at present what likely roles a promiscuous
β-chemokine receptor may play in either normal or pathologi-
cal situations. If this receptor is involved in chemokine-mediat-
ed chemoattraction then, assuming a relationship between
binding and signaling, it appears to be fairly indiscriminate in
the ligands to which it will respond. The expression of the
murine receptor on a range of cell types including primitive
hemopoietic cells, and the expression of the human homologue
on the primitive K562 cells and on U937 cells, suggest that it
may be a general chemoattractant receptor perhaps involved in
the general migration of cells around the body rather than in
the direct recruitment to inflammatory sites. However, the
high expression in solid organs such as the liver may indicate
that hD6 acts as a chemokine receptor for nonhemopoietic cells.
Evidence for these roles awaits the generation of hD6-/- mice,
which we are in the process of making.

hD6 as an HIV Co-receptor—The recent characterization of
the chemokine receptors CCR5 and CXCR4 as major co-recep-
tors with CD4 for entry of macrophage-tropic or T cell-tropic
strains of HIV-1, respectively, into host cells (see the Introduc-
tion), has prompted us to examine the ability of hD6 to act in
this manner. To this end we have generated a stably trans-
fected cell line Hos.CD4.hD6 that co-expresses the CD4 and
hD6 molecules on the cell surface. Expression of functional hD6
on these cells has been confirmed by Scatchard analysis, which
cloning of human D6

|        | Human | Murine |
|--------|-------|--------|
| MIP-1α | 64 nM | 920 pM |
| MIP-1β | 1.7 nM| 755 pM |
| RANTES | 3.6 nM| ND     |
| MCP1   | 16.5 nM| 615 nM |
| MCP2   | 768 pM| ND     |
| MCP3   | 1.2 nM| ND     |
| MCP4   | 5.97 nM| ND     |
| MCP5   | ND    | 6.3 nM |
| Eotaxin| 46 nM | 30 nM  |
| HCC1   | 27.2 nM| ND     |

* ND, not determined.
Acknowledgments—We thank Prof. John Wyke and Dr. Ian Pragnell for critical reading of this manuscript. Thanks are also due to Dr. Andrew Butler for providing cord blood leukocytes and Dr. Amanda Wilson for support services.

REFERENCES

1. Schall, T. J. (1991) Cytokine 3, 165–183
2. Oppenheim, J. J., Zachariae, C. O. C., Mukaida, N., and Matsushima K. (1991) Annu. Rev. Immunol. 9, 617–648
3. Kelner, G. S., Kennedy, J., Bacon, K. B., Kleyensteuber, S., Largaespada, D. A., Jenkins, N. A., Copeland, N. G., Bazan, J. F., Moore, K. W., Schall, T. J., and Zlotnik A. (1994) Science 266, 1395–1399
4. Donner, R., Muller, S., Entschladen, F., Schroder, J. M., Franke, P., Kraft, R., Fried, P., Clark-Lewis, I., and Kroczek, R. A. (1997) J. Biol. Chem. 272, 8817–8823
5. Bazan, J. F., Bacon, K. B., Hardiman, G., Wang, W., Soo, K., Rossi, D., Greaves, D. R., Zlotnik, A., and Schall T. J. (1997) Nature 385, 640–644
6. Murphy, P. M. (1996) Cytokine Growth Factor Rev. 7, 47–64
7. Murphy, P. M., and Tiffany, H. L. (1991) Science 253, 1280–1283
8. Holmes, W. E., Lee, J., Kuang, W.-J., Rice, G. C., and Wood, W. I. (1991) Science 253, 1278–1280
9. Loetscher, M., Gerber, M., Loetscher, P., Jones, S. A., Piali, L., Clark-Lewis, I., Baggioni, M., and Moser, B. (1996) J. Exp. Med. 184, 963–969
10. Bleul, C. C., Farzan, M., Choe, H., Parolin, C., Clark-Lewis, I., Sodorski, J., and Springer, T. A. (1996) Nature 382, 829–833
11. Oberlin, E., Amara, A., Bachelier, F., Beassa C., Virelizier, J.-L., Arentzana-Seisedos, F., Schwartz, O., Heard, J.-M., Clark-Lewis, I., Legler, D. F., Loetscher, M., Baggioni, M., and Moser, B. (1996) Nature 382, 833–835
12. Neote, K., DiGregorio, D., Mak, J. Y., Horuk, R., and Schall, T. J. (1993) Cell 72, 415–425
13. Charo, I. F., Myers, S. J., Herman, A., Franci, C., Connolly, A. J., and Coughlin, R. S. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2752–2756
14. Ponath, P. N., Qin, S., Post, T. W., Pan, W., Wu, L., Gerard, N. P., Newman, W., Gerard, C., and MacKay, C. R. (1996) J. Exp. Med. 183, 2437–2448
15. Combadiere, C., Ahuja, S. K., and Murphy, P. M. (1995) J. Biol. Chem. 270, 16491–16494
16. Power, C. A., Meyer, A., Nemeth, K., Bacon, K. B., Hoogewerf, A. J., Proudfoot, A. E. I., and Wells, T. N. C. (1995) J. Biol. Chem. 270, 19445–19500
17. Crotty, S., LeToux, J., Gao, J.-L., Pease, J., Locat, M., Combadiere, C., Moi, W., Bonner, T. I., and Murphy, P. M. (1997) J. Exp. Med. 186, 165–170
18. Sumter, R., Loetscher, M., Legler, D. F., Clark-Lewis, I., Baggioni, M., and Moser, B. (1997) J. Biol. Chem. 272, 17251–17254
19. Zhao-Hai, L., Zi-xuan, W., Horuk, R., Hesselgesser, J., Yan-chun, L., Hadley, T. J., and Peiper, S. C. (1995) J. Biol. Chem. 270, 26239–26245
20. Horuk, R., Zi-xuan, W., Peiper, S. C., and Hesselgesser, J. (1994) J. Biol. Chem. 269, 17730–17733
21. Gao, J.-L., and Murphy, P. M. (1994) J. Biol. Chem. 269, 28539–28542
22. Ahuja, S. K., Gao, J.-L., and Murphy, P. M. (1994) Immunol. Today 15, 251–257
23. Arvanitakis, L., Geras-Raaka, E., Varma, A., Gershengorn, M. C., and Cesarman, E. (1997) Nature 383, 347–350
24. Alkhatib, G., Combadiere, C., Breder, C. C., Feng, Y., Kennedy, P. E., Murphy, P. M., and Berger, E. A. (1996) Science 272, 1955–1958
25. Deng, H., Liu, R., Eilmeier, W., Choe, S., Unutmaz, D., Burkhart, M., DEmarzo, P., Marmion, S., Sutton, R. E., Hill, C. M., Davis, C. B., Peiper, S. C., Schall, T. J., Littman, D. R., and Landau, N. R. (1996) Nature 381, 661–666
26. Dragoo, T., Litwin, V., Allaway, G. P., Martin, S. R., Huang, Y., Nagashima, K. A., Cayanan, C., Maddon, P. J., Koup, R. A., Moore, P. J., and Paxton, W. A. (1996) Nature 381, 667–673
27. Ribas, R. J. B., Wylie, S. W., Pragnell, I. B., and Graham, G. J. (1997) J. Biol. Chem. 272, 12501–12504
28. Graham, G. J., Mackenzie, J., Lowe, S., Tsang, M. L.-S., Weatherbee, J. A., Issacson, A., Medicherla, J., Fang, F., Wilkinson, P. C., and Pragnell, I. B. (1994) J. Biol. Chem. 269, 4974–4978
29. Graham, G. J., Wilkinson, P. C., Ribas, R. J. B., Lowe, S., Kolset, S. O., Parker, A., Freshney, M. G., Tsang, M. L.-S., and Pragnell, I. B. (1996) EMBO J. 15, 6506–6515
30. Liu, R., Paxton, W. A., Choe, S., Ceradini, D., Martin, S. R., Horuk, R., MacDonald, M. E., Stuhlmann, H., Koup, R. A., and Landau, N. R. (1996) Cell 86, 367–377
31. Morgenstern, J. P., and Land, H. (1990) Nucleic Acids Res. 18, 3587–3596
32. Graham, G. J., Zhou, L., Weatherbee, J. A., Tsang, M. L.-S., Napolitano, M., Leonard, W. J., Pragnell, I. B. (1993) Cell Growth Differ. 4, 137–146
33. Munson, P. J., and Robard, D. (1980) Anal. Biochem. 107, 220–239
34. Farzan, M., Choe, H., Martin, K. A., Sun, Y., Sidelko, M., Mackay, C. R., Gerard, N. P., Sodroski, J., and Gerard, C. (1997) J. Biol. Chem. 272, 6584–6587
35. Savarese, T. M., and Fraser, C. M. (1992) Biochem. J. 283, 1–19
36. Sarafi, M. N., Garcia-Zepeda, E. A., MacLean, J. A., Chao, I. F., and Luster, A. D. (1997) J. Exp. Med. 185, 99–109