Isolation, Characterization, and cDNA Cloning of Chicken Turpentine-induced Protein, a New Member of the Scavenger Receptor Cysteine-rich (SRCR) Family of Proteins*

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Acute-phase serum proteins were induced by administering a chicken with turpentine oil. One of these proteins was a new protein that appeared in front of albumin in polyacrylamide disc gel electrophoresis using a 4.5–16% gel. To purify this protein, turpentine-administered chicken serum was fractionated by ammonium sulfate precipitation at 50% saturation, and the supernatant fraction was chromatographed on a DEAE-Toyopearl 650S column. The purified protein is a mannos-glycoprotein, and its N-terminal sequence, determined by the Edoman method, is not homologous from that of other reported acute-phase proteins. An analysis of physiological function with two different test systems, chemiluminescence measurement and electron spin resonance spectroscopy, showed that the purified protein has antioxidant activity and inhibits superoxide (O2) mediated by activation of the receptor. In support of these results, the complete amino acid sequence of 18-B is homologous to the scavenger receptor cysteine-rich (SRCR) family of proteins that participate in the regulation of leukocyte function. 18-B is composed of four SRCR domains, which is different from the previously characterized SRCR family of proteins such as Spa, CD6, and CD163. These findings indicate that turpentine-induced 18-B, a new member of scavenger receptor cysteine-rich family, may be implicated in regulation of cell function in a manner of inhibition of the overproduction of the reactive oxygen species.

Several acute-phase proteins in serum, such as C-reactive protein, hemopexin, fibrin, fibrinogen, transferrin, α1-acid glycoprotein, and α2-macroglobulin, have been reported in chicken (1–7). These proteins are present in normal serum, but their levels increase in inflammatory diseases. We have been searching for new marker proteins to improve the diagnosis for the acute phase of chicken. Turpentine enhances the synthesis of acute-phase proteins and has been used as an inducer of acute inflammation (8, 9).

Tohjo et al. (10) described two proteins, named 18-B and 18-C, that appeared in abundance after administration of turpentine. These proteins migrated in front of albumin in 4.5–16% polyacrylamide disc gel electrophoresis (PAGE).1 18-B was not detected in the serum of healthy chickens, the protein was considered to be a new acute-phase protein. Tohjo et al. (10) did not further characterize 18-B.

On the other hand, Urban et al. (11) reported that a new acute-phase α1-protein in rat was induced by turpentine administration and that its molecular mass based on SDS-PAGE was 68 kDa. 18-B in chicken is similar in electrophoretic characteristics and molecular size to Urban’s protein in rat, but it is not clear whether both these proteins are identical or not.

In this study, we attempted to identify a new acute-phase protein in chicken serum to establish a new marker for acute-phase inflammation. Thus, we purified a turpentine-induced protein from chicken serum and compared its molecular properties with those of the rat protein. In addition, we report the physiological function, cDNA cloning, and characteristics of 18-B.

EXPERIMENTAL PROCEDURES

Chicken Serum—An adult female Leghorn chicken was intramuscularly administrated with turpentine (3 ml/kg of body weight) and bled 48 h later. Serum was prepared and was stored at −20 °C before use. Isolation Procedure of 18-B—Saturated ammonium sulfate solution was added to the serum to 50% saturation, and the mixture was allowed to stand for 1 h at room temperature. A precipitate formed and was removed by centrifugation at 10,000 × g for 30 min at 10°C. The supernatant was dialyzed against 1,000 volumes of borax-phosphate buffer (20 mM Na2B4O7·10H2O and 60 mM NaH2PO4·2H2O, pH 8.1) overnight at 4°C. The dialyzed sample was applied to a DEAE-Toyopearl 650S column (0.8 × 25 cm, Tosoh Co., Tokyo, Japan) equilibrated with the same buffer. Proteins were eluted from the column by the linear gradient method with 0–0.5 M NaCl in borax-phosphate buffer at a flow rate of 1 ml/min. The eluate was monitored at 280 nm. The fraction corresponding to a peak that was not observed in the serum of healthy chickens was collected. The purified protein was identified as 18-B by its mobility in the gradient gel electrophoresis (12).

The abbreviations used are: PAGE, polyacrylamide disc gel electrophoresis; BSA, bovine serum albumin; ConA, concanavalin A; WGA, wheat germ agglutinin; UE4-A, ulex europeus agglutinin I; SBA, soybean agglutinin; DBA, dolichos biflorus agglutinin; RCA1, ricinus communis agglutinin I; PNA, peanut agglutinin; PAS, periodic acid-Schiff; HBSS, Hank’s balanced salt solution; MPO, phosphol 12-myristate 13-acetate; sOZ, serum-opsonized zymosan; O2, superoxide; Cu/Zn-SOD, copper- and zinc-containing superoxide dismutase; ESR, electron spin resonance; DEPMP0, 3-(diethoxyphosphoryl)-5-methyl-1-pyrroline N-oxide; PKC, protein kinase C; DMP0, 5,5-dimethyl-1-pyrroline N-oxide; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA end; bp, base pair(s); SRCR, scavenger receptor cysteine-rich.

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The nucleotide sequence reported in this paper has been submitted to the DDBJ/GenBank™/EMBL Data Bank with accession number AB051832.

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Chemical Analysis—Protein concentration was determined by the method of Lowry et al. (13) with BSA as the standard protein. The protein concentration in the column effluent was monitored by an UV monitor at a wavelength of 280 nm. Total carbohydrate content was determined by the phenol-sulfuric acid method with glucose as the standard (14). Sugar chains were analyzed using ConA, WGA, UEA-I, SBA, DBA, RCA-I, and PNA (Lectin Kit I, Vector Laboratories, Inc., Burlingame, CA).

Electrophoresis—SDS-PAGE was carried out by the method of Laemmli (15) using myosin, β-galactosidase, phosphorylase, BSA, ovalbumin, and carboxy anhydrase as standards. Disc electrophoresis was done according to the method of Sjöstedt et al. (12). Protein bands were stained with Coomassie Brilliant Blue R-250, and carbohydrate was stained with PAS reagent (16).

Immunological Assay—Double diffusion tests to assay the purity and antigenicity were done in 1% agarose in phosphate-buffered saline. Immunoblotting was performed by Towbin’s method (17). A rabbit anti-chicken whole serum antibody and a rabbit anti-chicken albumin antibody were purchased from ICN Pharmaceuticals, Inc. (Aurora, OH).

A gelatin- and horseradish peroxidase-conjugated horse anti-rabbit IgG (Bio-Rad) was used to detect the protein bands.

N-terminal Sequence Analysis—The protein 18-B was blotted to a polyvinylidene difluoride membrane (Immobilon Transfer Membranes, Nihon Millipore Ltd., Tokyo, Japan) after SDS-PAGE on a 7.5% gel. The N-terminal amino acid sequence was determined by Edman degradation with a model 477A protein sequencer on-line with a model 120A phenylthiohydantoin-derivative analyzer (PerkinElmer Life Sciences).

Preparations of Chicken Heterophils—Heterophils were isolated by means of a discontinuous gradient of 18 and 24% Ficoll (Ficoll 400, Amersham Pharmacia Biotech AB, Uppsala, Sweden) according to the established method (18). The isolated cells were suspended in Ca2+/Mg2+-free HBSS (Life Technologies, Inc.). Cell viability was always more than 95% by a trypan blue exclusion test.

Preparation of Stimulants—All the chemicals used below were purchased from Sigma. PMA and luminol were dissolved in dimethyl sulfoxide (Me2SO) at a stock concentration of 1 mg/ml and 100 mM, respectively. The final concentration of PMA was 1 μM. The final concentration of soZ was 1 μM. The preparation of 18-B was released in the high ionic strength phase as shown in the previous PCR product as a template. From the obtained 3'-cDNA sequence, two gene-specific reverse primers, P3 (5'-GGCCACGCCT-GACTAGTAC-3') and P4 (5'-AAGCGGACCACCTACGAGGTTGTTG-3') were designed for 3'-RACE. 3'-Reverse transcription was carried out with the outer primer P4 followed by RNase H treatment. The first strand cDNA was tailed at the 3'-end by terminal deoxynucleotidyl transferase (Life Technologies, Inc.) with dCTP and T4 RNA polymerase (Takara, Kyoto, Japan). DNA Sequencing—To determine the sequence of the amplified products, PCR products were subcloned into the pGEM-T easy vector (Promega Co., Madison, WI). DNA sequencing was performed on at least three independent PCR products in both directions by the dideoxynucleotide transferase (Life Technologies, Inc.) with dCTP and T7 or T3 RNA polymerase (Takara, Kyoto, Japan).

RESULTS

Identification—In the disc electrophoresis of chicken serum obtained after turpentine administration, two protein bands appeared in front of the albumin band (Fig. 1B). It was proved by 4.5–16% PAGE that the protein purified from the whole serum by salting out and DEAE chromatography corresponds to the 18-B protein reported by Tohjo et al. (10). To determine whether the protein, 18-B, could be detected in normal chicken serum and whether it was antigenically different from chicken albumin, Western blotting was carried out. The 18-B was not detectable in the normal serum (Fig. 2, lane 1), but the purified materials positively reacted with rabbit anti-chicken whole serum (Fig. 2B, lane 2). With anti-chicken albumin, only albumin, but not 18-B, was immunologically stained (Fig. 2C).

Identification of 18-B—The protein bands in the turpentine-treated chicken were separated by DEAE chromatography (Fig. 3). When the column was eluted by the linear gradient method, 18-B was released in the high ionic strength phase as shown in

Fig. 1. Appearance of the new protein 18-B in acute-phase inflammation. Normal serum and serum from a turpentine-administered chicken were electrophoresed on a 4.5–16% gradient gel. A, normal chicken serum. B, chicken serum obtained 48 h after intramuscular administration of turpentine. As shown in B, two peaks, 18-B and 18-C, appeared in front of 18-A (albumin).
Fig. 2. Identification of 18-B by immunoblotting with rabbit anti-chicken serum and rabbit anti-chicken albumin serum. A, protein staining with Coomassie Brilliant Blue R-250. Lane 1, albumin; lane 2, purified 18-B. B, immunoblotting with rabbit anti-chicken serum. Lane 1, normal chicken serum; lane 2, purified 18-B. C, immunoblotting with rabbit anti-chicken albumin serum. Lane 1, albumin; lane 2, purified 18-B. 18-B was not detected in the normal serum and was different from albumin in antigenicity.

Fig. 3. Elution profile of proteins on DEAE chromatography with a DEAE-Toyopearl-650S column. 18-B was eluted in the third-peak without contamination of other proteins.

Molecular Weight Determination—The SDS-PAGE pattern indicated that 18-B contains a single peptide, and its molecular size is 54 or 66 kDa in the absence or presence of 2-mercaptoethanol, respectively (Fig. 4, lanes 2 and 3).

Carbohydrate Constitution—18-B showed a positive reaction in PAS staining (Fig. 5A). A phenol-sulfuric acid assay indicated that 18-B contains ~28.6% (w/w) carbohydrate. 18-B was stained with ConA only, which is specific for terminal mannosese residues, but it was not stained by WGA, UEA-I, SBA, DBA, RCA-I, or PNA (Fig. 5B). These results indicate that 18-B is a glycoprotein with mannosese.

N-terminal Sequence—The N-terminal amino acid sequence determined by Edman method is shown in the gray highlighted areas of Fig. 8A. Primers for initial PCR were designed in this region.

Effects of 18-B on the Luminol-enhanced Chemiluminescence in PMA- and sOZ-stimulated Heterophils—To study the involvement of 18-B in the functions of avian heterophils, we measured luminol-enhanced chemiluminescence from PMA-stimulated heterophils. PMA is known to directly stimulate PKC, to phosphorylate NADPH oxidase component such as p47phox and then activate NADPH oxidase to produce O_2^- (19–21). When the cells were stimulated by PMA, the chemiluminescence rapidly increased in the first 10 min and then gradually decreased (closed circle of Fig. 6A). The PMA-induced chemiluminescence was inhibited by Cu/Zn-SOD (open square) but not by BSA (closed triangle). Thus, the PMA-induced chemiluminescence is due to O_2^- production from the heterophils. When the cells were stimulated by PMA in the presence of 18-B, a small reduction of the chemiluminescence was observed. About 70–80% of the control peak counts was preserved, even in the presence of 18-B. Next, to investigate receptor-mediated O_2^- production from the heterophils (22), sOZ was employed. The chemiluminescence evoked by sOZ (the control) was relatively low, but the kinetics of the increase of the chemiluminescence was similar to that in PMA stimulation (closed circles of Fig. 6B). The treatment of 18-B induced a dose-dependent decrease in sOZ-evoked chemiluminescence of heterophils (Fig. 6B). In contrast to PMA stimulation, the inhibition of the chemiluminescence of sOZ-stimulated cells by 18-B was significantly greater than that of PMA-stimulated cells. For example, in the presence of the same concentration of 18-B (5 μg/ml), the chemiluminescence peaks evoked by sOZ and PMA were 70 and 25% of the control values, respectively.

Spin-trapping Detection of the Oxygen Radicals in PMA- and sOZ-stimulated Heterophils and the Effects of 18-B on the O_2^- Production—In preliminary experiments, we tried ESR spin-trapping using DMPO, which is known to be useful for detecting oxygen radicals in phagocytes (23, 24). These experiments were unsuccessful, possibly because the amount of O_2^- produced from heterophils was quite low and because the DMPO spin-
adducts was unstable (having half-lives of only a few minutes). Therefore, in this experiment, we used a new spin trap, the β-phosphorylated nitrotr DEPMPO. DEPMPO is much more stable than the DMPO adduct, having a 15-fold longer half-life. Fig. 7B shows the ESR signals when the heterophils were stimulated for 20 min by PMA in the presence of DEPMPO. Similarly, Fig. 7E shows the ESR signals when the heterophils were stimulated for 40 min by sOZ. These ESR signals were quite similar to those reported by Chamulitrat (25) and consisted of two signals due to DEPMPO-OOH and DEPMPO-OH. Both signals were completely inhibited by the presence of Cu/Zn-SOD. These results indicated that the DEPMPO-OOH and DEPMPO-OH adducts were produced by O$_2^-$ in PMA- and sOZ-stimulated heterophils. The components indicated by the asterisk and open circle were chosen for comparison, as the component due to DEPMPO-OOH (asterisk) did not overlap with that of DEPMPO-OH (open circle). Treatment of sOZ-stimulated heterophils with 18-B completely inhibited production of DEPMPO-OOH (asterisk in Fig. 7, E and F), although the reduction of PMA-induced DEPMPO-OOH by 18-B was small (asterisk in Fig. 7, B and C).

cDNA Cloning of 18-B—We obtained the cDNA sequence of 18-B from a combination of PCR products as described under “Experimental Procedures” (Fig. 8A). The 1,410-bp open reading frame was followed by a 244-bp 3′-untranslated region. A potential polyadenylation signal was located 16 bp upstream of the poly(A) tail. The open reading frame encoded a protein with 470 deduced amino acids. There are two potential sites for N-linked glycosylation. The N-terminal region of deduced protein had the features of a secreted protein, which contained 20 hydrophobic amino acids. This hydrophobic peptide was followed by four cysteine-rich domains, each ~100 amino acids in length. These domains are significantly homologous to the cysteine-rich domains found in the SRCR group B family of proteins (Fig. 8B).

**DISCUSSION**

In this study, 18-B was purified by salting-out and DEAE chromatography, finally yielding 0.4 mg of 18-B from 1 ml of the chicken acute-phase serum. This protein corresponds to 18-B reported by Tohjo et al. (10) and was first isolated in the present study. This protein is a single peptide with a molecular mass of 54 kDa.

Several chicken serum proteins, such as C-reactive protein, hemopexin, fibrin, fibrinogen, transferrin, α$_1$-acid glycoprotein, and α$_2$-macroglobulin, are shown to increase with increasing degree of inflammation (1–7). In addition, haptoglobin (9), transferrin (10), and α$_1$-acid glycoprotein (6) are shown to be elevated in chicken by administration of turpentine. However, none of these proteins had all the molecular properties of 18-B (molecular size, N-terminal sequence, carbohydrate composition, and antigenicity).

Urban et al. (11) reported a novel turpentine-induced glycoprotein with a single peptide chain in rat. This protein has a molecular mass of 56 kDa and a pI value of 4.7. These properties are similar to those of 18-B, but the N-terminal amino acid sequences of these two proteins are not homologous. These results suggest that 18-B is a new turpentine-induced protein of chicken.

To further understand the function of 18-B, the complete amino acid sequence was determined by cDNA cloning. The
deduced amino acid sequence of 18-B has four repeated sequences. A search of all sequences in the GenBank™ data base was carried out to identify similar sequences, which resulted in homology with members of the SRCR family of proteins, such as hensin, macrophage scavenger receptor, WC1, CRP-ductin, ebnerin, CD163, Spa, CD5, and CD6. There are two types of SRCR family: SRCR group A domains contain six cysteine residues and are encoded by two exons, which include macrophage scavenger receptor and related proteins (26), etc. SRCR group B domains contain eight cysteine residues and are encoded by a single exon, which include leukocyte antigens CD5, CD6, CD163 (M130), WC1, and Spa, etc. (27–31). Each of the cysteine-rich domains of 18-B shares high degrees of sequence homology with that of SRCR group B family, in particular conserved sequence elements, including eight cysteine residues. These indicate that 18-B belongs to the SRCR group B family. The domain organization of 18-B is similar to, but different from, those of the previously reported proteins. Because 18-B is composed exclusively of SRCR domains, it can be distinguished from multidomain proteins that have both CUB (C1r/C1s Uegf Bmp 1) and ZP (zona pellucida) domains besides SRCR domain, such as hensin, CRP-ductin, and ebnerin (32–34). Furthermore, 18-B has neither transmembrane nor cytoplasmic domain, which indicates that 18-B is different from membrane proteins, such as CD5, CD6, and CD163 (27, 35, 29). Secreted protein, Spa, is the only protein that has such a simple domain organization, which is composed of SRCR domain only (31). However, comparison of 18-B with Spa revealed two major differences: the primary structure of 18-B is longer by 123 residues than that of Spa, and 18-B has four domains, whereas Spa has three SRCR domains. On the basis of these findings, we propose that 18-B is a new member of the SRCR group B family of proteins.

Leukocyte function is regulated by a discrete number of cell surface and secreted antigens that govern leukocyte activation, proliferation, survival, cell adhesion and migration, and effector function. Among the proteins that have been shown to regulate leukocyte function is the SRCR family of proteins.

**Fig. 8.** A, deduced amino acid sequence of 18-B cDNA. Putative signal peptide is double underlined. SRCR domains are underlined. Potential N-glycosylation site is bold. Polyadenylation site is italic. The N-terminal sequence determined previously by Edoman method is indicated by the gray highlighted areas. B, comparison of the domain structure of SRCR family proteins. Gray highlighted areas are regions 15 out of 19 amino acids are homologous. C, domain organization of 18-B, Spa, CD6, and CD163. TM, transmembrane; CT, cytoplasmic region.
The production of heterophils is mostly by activation of receptor and not by direct activation of PKC. The function of members of the SRCR group B family is not defined conclusively and fully understood yet. WC1 is involved in T cell regulation (30). CD5 and CD6 modulate T cell function. CD5 binds to CD72 (40), CD6 to ALCAM (39). These proteins obviously exert their functions after binding to activation. 18-B is a new member of SRCR family of proteins, which is a 18-B-binding protein on heterophils, and 18-B may regulate heterophil function in a manner of inhibition of the over-production of the reactive oxygen species.

In conclusion, our studies demonstrate that turpentine-induced 18-B is a new member of SRCR family of proteins, which may be implicated in regulation of cell function.

REFERENCES

1. Patterson, L. T., and Mora, E. C. (1965) Tex. Rep. Biol. Med. 23, 600–606
2. Grieninger, G., Liang, T. J., Beuving, G., Mauzy-Melitz, D., Goldfarb, V., Metcalfe, S. A., and Muller-Eberhard, U. (1986) J. Biol. Chem. 261, 15719–15724
3. Amrani, D. L., Mauzy-Melitz, D., and Mosesson, M. W. (1986) Biochem. J. 238, 365–371
4. Grieninger, G., Oddoux, C., Diamond, L., Weissbach, L., and Plant, P. W. (1989) Ann. N. Y. Acad. Sci. 557, 257–270
5. Hallquist, N. A., and Klaing, K. C. (1994) Comp. Biochem. Physiol. Biochem. Mol. Biol. 108, 375–384
6. Delers, F., Domingo, M., and Engler, R. (1983) Comp. Biochem. Physiol. 74, 619–622
7. Klaing, K. C. (1991) Poult. Sci. 70, 1176–1186
8. Sinha, B. K., Vegad, J. L., and Awadhiya, R. P. (1987) Res. Vet. Sci. 42, 365–372
9. Jain, N. K., Vegad, J. L., and Awadhiya, R. P. (1982) Vet. Rec. 110, 421–422
10. Tohjo, H., Miyoshi, F., Uchida, E., Niiyama, M., and Syuto, B. (1995) Poult. Sci. 74, 648–655
11. Urban, J., Chan, D., and Schreiber, G. (1979) J. Biol. Chem. 254, 10565–10568
12. Syuto, B., Miyake, Y., and Kabo, S. (1981) Jpn. J. Vet. Sci. 43, 71–77
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
14. Dubois, M., Giles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956) Anal. Chem. 28, 350–356
15. Laemmli, U. K. (1970) Nature 227, 680–685
16. Finley, C. W., and Klasing, K. C. (1994) Comp. Biochem. Physiol. 108, 9539–9543
17. Inanami, O., Johnson, J. L., and Babior, B. M. (1998) Arch. Biochem. Biophys. 350, 36–40
18. Yamamoto, T., Inanami, O., Nagahata, H., Cui, Y., and Kuwabara, M. (2000) FEBS Lett. 467, 253–258
19. Britigan, B. E., Cohen, M. S., and Rosen, G. M. (1987) J. Leukoc. Biol. 41, 349–362
20. Britigan, B. E., Rosen, G. M., Chai, Y., and Cohen, M. S. (1986) J. Biol. Chem. 261, 4426–4431
21. Chamaliirat, W. (1999) Free Radiac. Biol. Med. 27, 411–421
22. Kodama, T., Freeman, M., Rohrer, L., Zahrecky, J., Matsudaira, P., and Krieger, M. (1990) Nature 343, 531–535
23. Jones, N. H., Clabby, M. L., Dialynas, D. P., Huang, H. J., Herzenberg, L. A., and Strominger, J. L. (1984) Nature 313, 348–349
24. Huang, H. J., Jones, N. H., Strominger, J. L., and Herzenberg, L. A. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 204–208
25. Law, S. K., Mckimm, K. J., Shaw, J. M., Zong, X. P., Dong, Y., Willis, A. C., and Mason, D. Y. (1993) Eur. J. Immunol. 23, 2320–2325
26. Cheng, H., Bjerkes, M., and Chen, H. (1996) Anat. Rec. 244, 327–343
27. Fujita, K., and Nishimoto, M. J., MacHugh, N. D., Morrison, W. L., and Clevers, H. C. (1992) J. Immunol. 149, 3273–3277
28. Gebe, J. A., Kiener, P. A., Ring, H. Z., Li, X., Francke, U., and Aruffo, A. (1997) J. Biol. Chem. 272, 6151–6158
29. Takito, J., Yan, L., Hikita, C., Vijayakumar, S., Warburton, D., and Al-Awqati, Q. (1999) Am. J. Physiol. 277, F277–F289
30. Cheng, H., Bjerkes, M., and Chen, H. (1996) Anat. Rec. 244, 327–343
31. Li, X. J., and Snyder, S. H. (1995) J. Biol. Chem. 270, 17674–17679
32. Aruffo, A., Melnick, M. B., Lines, P. S., and Seed, B. (1991) J. Exp. Med. 174, 949–952
33. Krieger, M. (1997) Curr. Opin. Lipidol. 8, 275–280
34. Aruffo, A., Bowen, M. A., Patel, D. D., Haynes, B. F., Starling, G. C., Gebe, J. A., and Bajorath, J. (1997) Immunol. Today 18, 498–504
35. Vijayakumar, S., Takito, J., Hikita, C., and Al-Awqati, Q. (1999) J. Cell Biol. 144, 1057–1067
36. Whitney, G. S., Starling, G. C., Bowen, M. A., Modrell, B., Siadak, A. W., and Aruffo, A. (1995) J. Biol. Chem. 270, 18187–18190
37. van de Velde, H., van Hoogen, I., Luo, W., Parves, J. R., and Thieleman, K. (1991) Nature 351, 662–665