Volume 335, number 1, 51–56 FEBS 13290 November 1993

Alternative transcripts of the human CD23/FcεRII

A possible novel mechanism of generating a soluble isoform in the type-II cell surface receptor

Minoru Matsui[a,*], Rafael Nunez[b], Yoshihumi Sachi[a], Richard G. Lynch[b], Junji Yodoi[a]

[a]*Institute for Virus Research, Kyoto University, 53 Kawahara-cho, Sakyo-ku, Kyoto 606, Japan*
[b]*Department of Pathology and Microbiology, University of Iowa, Iowa City, IA 52242, USA*

Received 13 September 1993

Human CD23/FcεRII is a 45 kDa type-II membrane glycoprotein having two isoforms (a and b) that only differ in the structures of their intracytoplasmic tails. CD23/FcεRII has been demonstrated to have multiple roles in the immune system such as regulation of lymphocyte growth and differentiation and IgE-mediated immune responses. Here, we found that the human B-cell line RPMI8866, in addition to a and b transcripts, contained shorter transcripts (a′ and b′) that lack the entire third exon. These alternative transcripts were also detected in peripheral blood lymphocytes as well as other hematopoietic cell lines with CD23/FcεRII. Because exon 3 encodes all of the transmembrane segment and the anchoring region of the cytoplasmic tail, it is suggested that a′ and b′ transcripts encode secretory forms of CD23/FcεRII or they may function as regulatory transcripts involved in the control of CD23/FcεRII expression.

CD23/FcεRII; Type-II cell surface receptor; C-type animal lectin; Alternative splicing; Soluble isoform

1. INTRODUCTION

Human CD23/FcεRII is a low affinity Fc receptor for IgE and plays various biological roles [1]. It is expressed on μ/δ positive B lymphocytes [2,3,4] as well as some T lymphocytes [5] and macrophages [6]. So far, two alternative spliced human CD23/FcεRII isoforms, namely a and b, have been demonstrated and their distributions and functions were intensively studied [7,8]. Type a and b CD23/FcεRII differ in their cytoplasmic tails by the use of different initiating exons. Several different forms of soluble CD23/FcεRII are cleaved from the extracellular domain [9] and some of them have been reported to be involved in regulation of immune-system functions such as proliferation of bone marrow-derived T cells [10] and survival of germinal center B cells [11]. Unlike other Fc receptors which belong to the immunoglobulin superfamily, CD23/FcεRII shows homology to C-type animal lectins such as the mannose binding protein. Recently CD23/FcεRII has also found to be a natural ligand of CD21/complement receptor 2 [12].

In the present studies we have determined that two additional transcripts of CD23/FcεRII exist. These novel transcripts lack their entire third exons, regions which encode the anchoring and transmembrane domains [13], except for the new joint between exon two and four, there are no changes in sequences of their amino acid reading frames. We analyzed the property of these transcripts in particular relation to formation of soluble receptor.

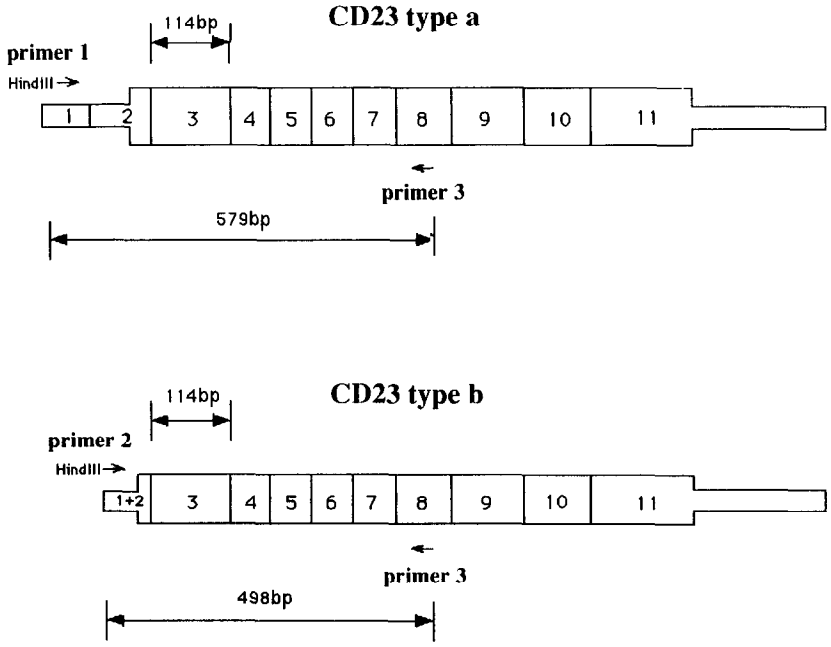
*Corresponding author. Fax: (81) (75) 761-5766.

2. MATERIALS AND METHODS

Total RNA was purified from the human EBV-transformed B cell line RPMI8866 and COS cells using guanidinium isothiocyanate and CsCl centrifugation. Single strand cDNA was synthesized using First-Strand cDNA Synthesis Kit (Pharmacia LKB Biotechnology, Uppsala, Sweden) following the manufacturer's instructions. Oligonucleotide sequences of the primers used in the PCR are shown in Fig. 1. PCR fragments were subcloned into pSP72 plasmid (Promega, Madison, WI) and sequenced using Sequenase (USB, Cleveland, OH) following the manufacturer's instructions. The ribonuclease protection assay was performed using RPA II kit (Ambion, Austin, TX) following the manufacturer's instructions. Electroporation was performed using GenePulser (BioRad) under the condition of 0.4 kV, 125 μF. Cell lysates were prepared by treating cells with solubilizing buffer containing 0.5% NP-40 for 30 min. Sandwich ELISA for the detection of CD23/FcεRII was performed as previously described with a slight modification [5]. In this ELISA study, we used anti-CD23/FcεRII mAb H107 and E70. Immunoblotting analysis was carried out by the method described previously [14].

3. RESULTS

RT-PCR was performed to isolate human CD23/FcεRII N-terminal region cDNA using primers recognizing the 5′ untranslated region of either type a or b CD23/FcεRII and a common extracellular region (Fig. 1). Unexpectedly, both primer pairs generated two



primer 1 5'- cgaagctttctggtcgaccccaacacactag -3'

primer 2 5'- cgaagcttgcggggacgcaatagagtcagag -3'

primer 3 5'- ctcccggagtctttccagcaaatctg -3'

Fig. 1. Design of PCR primers for CD23/FcεRII. Primer 1 is designed for exon 1, which is specific for type a CD23/FcεRII. Primer 2 is for exon 1 + 2 which is specific for type b CD23/FcεRII. Primer 3 is for exon 8, which is common to both CD23/FcεRII isoforms. *Hin*dIII sites (underlined) are artificially introduced in primer 1 and 2 at their 5′ end for further subcloning. The amplified type a and b CD23/FcεRII are expected to be the size of 579 and 498 base-pairs, respectively.

bands, one of the expected size, and a second approximately 100 bp smaller (Fig. 2).

To determine the relationship between these two products, they were purified and subcloned in pSP72 vector and sequenced. As can be seen in Fig. 3, the nucleotide sequences of the larger fragments were exactly the same as either type a or b CD23/FcεRII which have been already reported. The sequence of the smaller fragments were also identical with those of type a or b CD23/FcεRII, except that they do not contain the entire third exon.

To further confirm the existence of these novel transcripts, we performed a ribonuclease protection assay using four riboprobes (Fig. 4). A significant amount of the predicted isoform was detected, but there were several additional bands whose origins were not clear. Because these data are reproducible, they may reflect additional transcripts or ribonuclease sensitive sites probably due to their secondary structures.

To identify the characteristics of the putative proteins translated from these two novel transcripts, we constructed both type a and b CD23/FcεRII cDNA without the third exon, and designated these as type a′ and type b′ CD23/FcεRII, respectively. COS-7 cells were transfected with these four types of CD23/FcεRII cDNAs under the control of the SRa promoter. The ribonuclease protection assays using type a CD23/FcεRII riboprobes revealed that there were almost the same amount of introduced transcripts in these cells (Fig. 5). As for proteins, however, we only detected type a and b CD23/FcεRII using surface staining, immunoblotting and enzyme-linked immunosorbent assays (Fig. 6). We further introduced these cDNAs stably in a human lymphoid cell line, YT, but type a′ and b′ protein have not been detected so far, while type a and b are expressed successfully (data not shown).

4. DISCUSSION

We have identified skipping of exon 3 in both type a and b CD23/FcεRII mRNA using RT-PCR and ribonuclease protection assays. In fact, it has been already noted by another group that the S1-nuclease protection analysis of CD23/FcεRII showed unexplained bands [7], which are now considered to be derived from the novel isoforms presented here. Though the two splicing

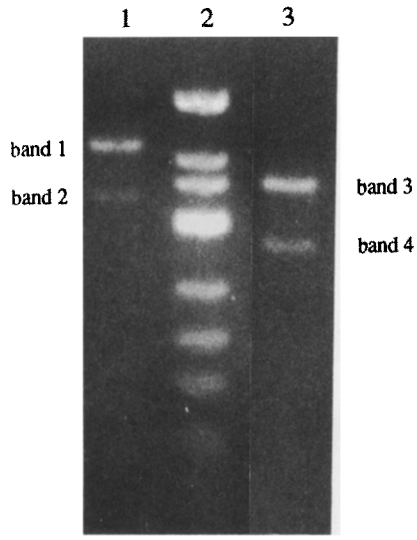


Fig. 2. RT-PCR analysis of CD23/FcεRII transcripts. Total RNA was extracted from RPMI8866 cells using GTC method and CsCl centrifugation. Complementary DNA was generated from 0.5 μg of the RNA and was used as templates for the PCR amplification (25 cycles). The products were analyzed with agarose gel electrophoresis. Lane 1 and lane 3 show the product using primers 1 and 3, primers 2 and 3, respectively. Bands 1 and 3 were expected CD23/FcεRII fragments but bands 2 and 4 had not been expected. Lane 2 shows DNA marker (ϕX174/*Hin*fI digest).

donor sites are different, both can use the same sequence just before the third exon as acceptor. In our RT-PCR, we could not detect other types of splicing. Because exon 3 is 114 base pairs long, this deletion causes only one junctional amino acid change while preserving the following amino acid reading frame. Further preliminary RT-PCR analysis demonstrated these novel transcripts in peripheral blood lymphocytes and in other hematopoietic cell lines with CD23/FcεRII and we are now investigating their quantitative changes under various conditions. We also detected skipping of the transmembrane segment in transcript of murine CD23/FcεRII [15]. These observations, together with the same findings with murine and human CD23/FcεRII, suggests that deletional splicing of exon 3 may have a biological importance rather than being a nonfunctional aberrant splicing.

A considerable number of cell surface receptors such as neural cell adhesion molecule [16], growth hormone receptor [17], interleukin-4 receptor [18] and fibroblast growth factor receptor [19] have been found to produce a soluble isoform using alternative splicing. A common structural feature of those receptors is a signal peptide at their 5′ end and their N-terminus outside and C-terminus inside the cell. In those receptors, alternative splicing which introduces a stop codon just upstream of the transmembrane segment results in the deletion of the following amino acids without affecting the extracellular segment. It seems particularly important for those spliced products to preserve a signal sequence for their efficient expression of protein. On the contrary, in the case of reverse-oriented type-II cell surface receptors such as CD23/FcεRII, introducing a stop codon upstream of the transmembrane segment results in the loss of the entire extracellular segment, so this mechanism would not produce a soluble isoform. Instead, it is plausible that deletional splicing of the transmembrane-coding exon(s), as we report here for human CD23/FcεRII, generates a soluble isoform, because this mechanism maintains the extracellular segment. However, as far as we know, such a mechanism has never been reported yet and this case probably is the first example.

In addition, because one of the type-II transmembrane proteins, invariant chain, was known to function in the cytoplasm as a transporter of MHC class II molecules [20], it is also needed to see whether these novel CD23/FcεRII isoforms are present intracellularly.

Although the ribonuclease protection assay documents that the a′ and b′ isoforms of CD23/FcεRII occur at the level of transcripts both in RPMI8866 and in COS transfectants, immunoblotting and ELISA have been unsuccessful in detecting the exon 3 deleted CD23/FcεRII at the protein level. Although we cannot completely exclude the possibility of the protein expression

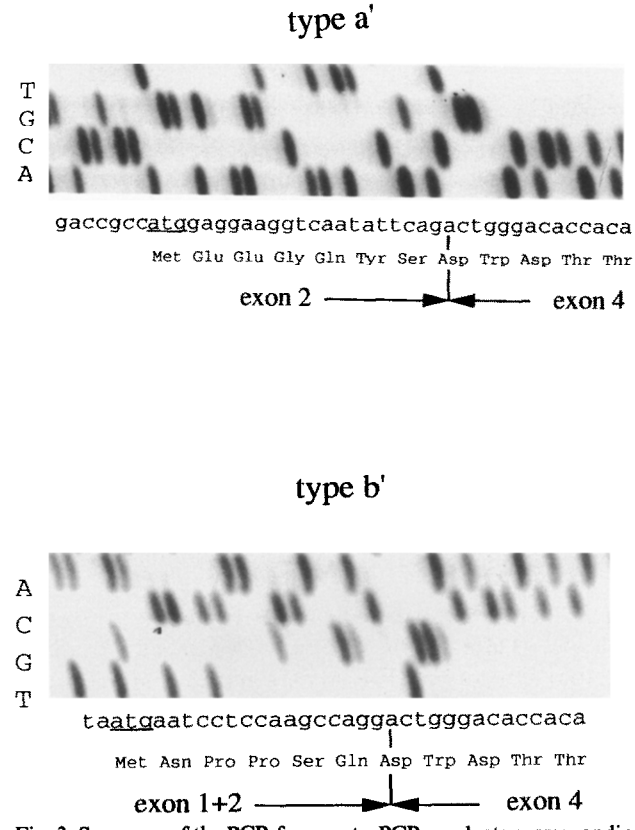


Fig. 3. Sequence of the PCR fragments. PCR products corresponding to the band 2 and band 4 in Fig. 2 were purified from the gel and subcloned into pSP72 plasmids and sequenced. These sequence data showed absence of the entire third exon. This change did not affect the reading frame. We designated these new fragments as type a′ and b′ CD23/FcεRII isoforms, respectively.

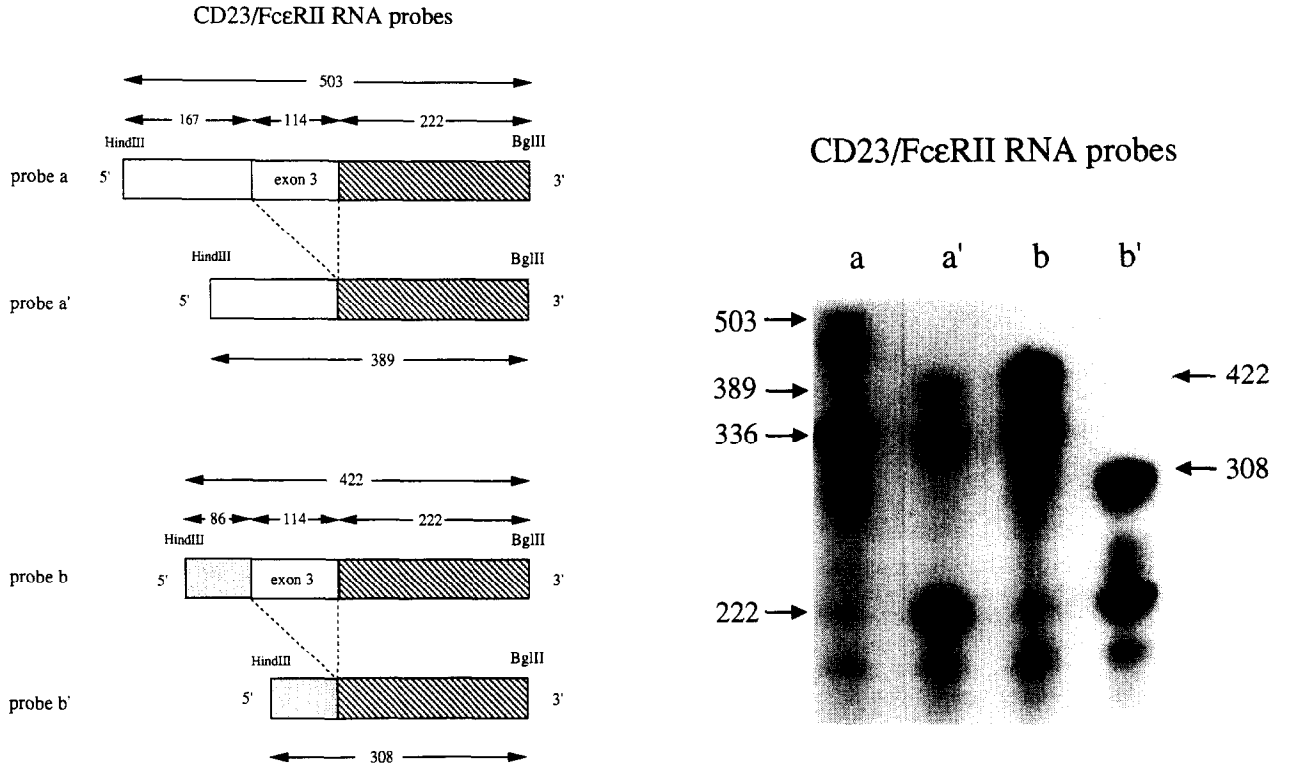
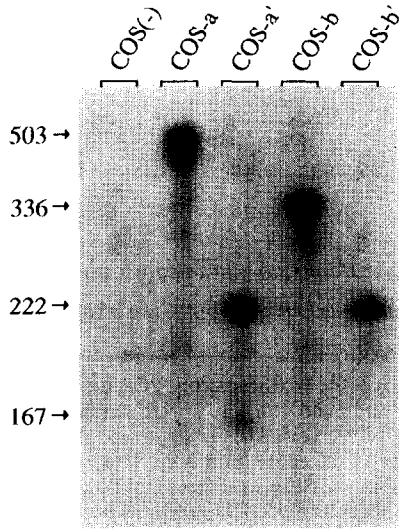


Fig. 4. Ribonuclease protection assay on RPMI8866. Total RNA (10 μg) prepared from RPMI8866 was annealed to each single strand probe, which contained the sequence corresponding to the *Hin*dIII-*Bgl*II fragments of the type a, a', b and b' CD23/FcεRII cDNA and then digested with RNase A and RNase T1. The size of protected bands were analyzed by polyacrylamide gel electrophoresis. The 222 base-pairs bands in lane a and lane b are derived from a' and b' RNA. The 389 base-pairs band in lane a' and the 308 base-pairs band in lane b' is derived from type a' and b' RNA, respectively.

in different circumstances, a possible explanation for the discrepancy between mRNA and protein expression is that absence of an N-terminal signal peptide in C-type animal lectins such as CD23/FcεRII, dictates that the substitutive hydrophobic transmembrane segment is necessary for post-translational translocation.

Alternatively, the truncated a' and b' transcripts might themselves be regulatory elements for expression of the full-length CD23/FcεRII protein through a novel mechanism. It is probable that the skipping of exon 3 converts full-length CD23/FcεRII mRNA into unstable transcripts and this decreases the expression of full-length protein. A second possibility is that the full-length mRNA stabilities are negatively-correlated with those of truncated ones through competition for a common degradation pathway. In either case exon 3 deletional splicing would influence CD23/FcεRII expression.

It might be biologically real that CD23/FcεRII is



←

Fig. 5. Identification of introduced CD23/FcεRII isoform transcripts. Type a, b, a' and b' CD23/FcεRII isoforms are introduced under the control of the SRα promoter. COS-7 cells were transfected with 10 μg of each expression vector by electroporation using GenePulser (Bio-Rad). After 48 hours, total RNA (10 μg) were prepared from these COS cells and ribonuclease protection assays were performed using type a CD23/FcεRII riboprobes as in the legend of Fig. 4. The 503 base-pairs band in lane COS-a, the 222 and 167 base-pairs bands in lane COS-a', the 336 base-pairs band in lane COS-b and the 222 base-pairs band in lane COS-b' are derived from the introduced CD23/FcεRII isoform transcripts. The parental COS cell did not give these bands as shown in lane COS(−). These results were also confirmed by the ribonuclease protection assays using type a', b and b' riboprobes (data not shown).

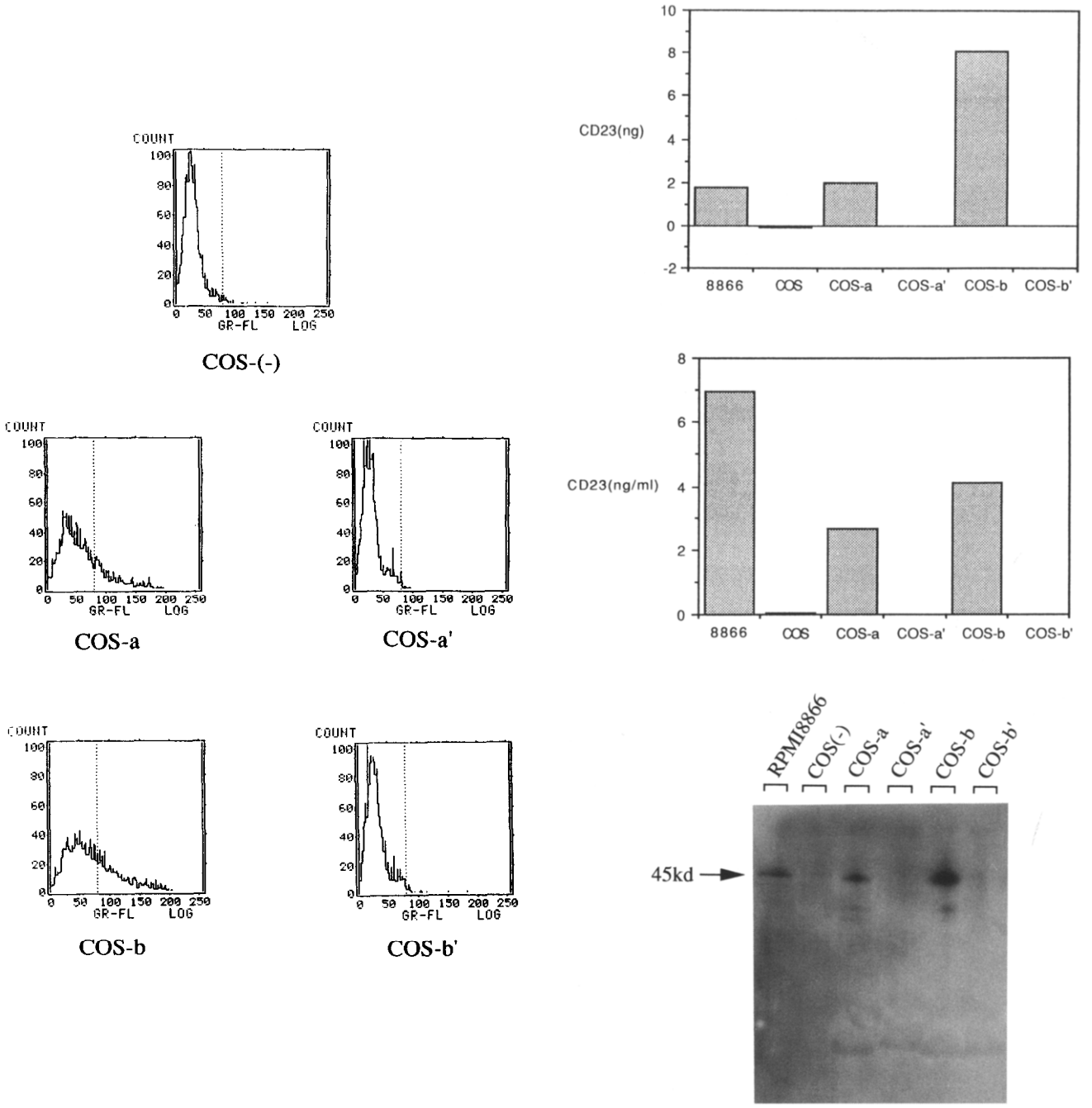


Fig. 6. Protein expression of the introduced CD23/FcεRII isoforms. Type a, b, a′ and b′ CD23/FcεRII isoforms are introduced into COS-7 cells as described in the legend of Fig. 5. After 72 h, the cells were harvested and assayed for the level of CD23/FcεRII protein expression. (a) Surface staining. The cells were stained with anti-CD23/FcεRII monoclonal antibody H107 and secondary FITC-labeled antibody and analyzed using flowcytometry. COS cells transfected with PBS (negative control) are also shown. (b,c) Sandwich ELISA. (b) Cell lysate or (c) Cell supernatant were assayed with sandwich enzyme-linked immunosorbent assay using two different monoclonal antibodies against CD23/FcεRII. The amount of CD23/FcεRII in cell lysate is indicated as ng/50 μg of total protein. Data from RPMI8866 and PBS transfected COS-7 cells are also shown as positive and negative controls. (d) Immunoblotting analysis. Lysates of COS cells and RPMI8866 solubilized in 0.5% Nonidet P-40 were electrophorased on a 15% polyacrylamide gel under nonreduced conditions. After electro-transfer to a polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA), the membrane was blocked with 2% BSA, and incubated with mAb H107. Horseradish peroxidase labelled anti-mouse IgG was used as the secondary antibody. The bands reactive with antibodies were visualized by horseradish peroxidase-ECL method (Amersham, Buckinghamshire, UK). Though both type a and b CD23/FcεRII were clearly detected, type a′ and b′ could not be found with all these methods.

regulated through multiple mechanisms including alternative mRNA splicing as well as proteolytic cleavage of extracellular portion of intact surface molecule. Further studies are needed to investigate whether co-expression of the alternatively spliced CD23/FcεRII alters the activity or function of the full-length receptor.

Acknowledgements: We thank Marita Robinson for technical assistance, Dr. Toshihiko Namba for development of the sandwich ELISA and Dr. Akira Shimizu for scientific advice.

REFERENCES

[1] Lynch, R.G., Sandor, M., Nunez, R., Mathur, A., Hagen, M., Waldschmidt, T., Ness, V.B., Nelms, K., Noben, N., Ibraghimov, A., Mordue, D., Sacco, R., Teeraratkul, P., Schaiff, W.T. and Iakoubov, L. (1992) Immunobiology 185, 235–2675.

[2] Kikutani, H., Inui, S., Sato, R., Barsumian, E.L., Owaki, H., Yamasaki, K., Kaisho, T., Uchibayashi, N., Hardy, R.R., Hirano, T., Tsunasawa, S., Sakiyama, F., Suemura, M. and Kishimoto, T. (1986) Cell 47, 657–665.

[3] Ikuta, K., Takami, M., Kim, C.W., Honjo, T., Miyoshi, T., Tagaya, Y., Kawabe, T. and Yodoi, J. (1987) Proc. Natl. Acad. Sci. USA 84, 819–823.

[4] Ludin, C., Hofstetter, H., Sarfati, M., Levy, C.A., Suter, U., Alaimo, D., Kilchherr, E., Frost, H. and Delespesse, G. (1987) EMBO J. 6, 109–114.

[5] Kawabe, T., Maekawa, N., Maeda, Y., Hosoda, M. and Yodoi, J. (1991) J. Immunol. 147, 548 553.

[6] Maekawa, N., Satoh, S., Kawabe, T., Maeda, Y., Hosoda, M., Iwata, S., Nakamura, K., Klein, E., Okada, H. and Yodoi, J. (1993) Mol. Immunol. (in press).

[7] Yokota, A., Kikutani, H., Tanaka, T., Sato, R., Barsumian, E.L., Suemura, M. and Kishimoto, T. (1988) Cell 55, 611–618.

[8] Yokota, A., Yukawa, K., Yamamoto, A., Sugiyama, K., Suemura, M., Tashiro, Y., Kishimoto, T. and Kikutani, H. (1992) Proc. Natl. Acad. Sci. USA. 89, 5030–5034.

[9] Letellier, M., Sarfati, M. and Delespesse, G. (1989) Mol. Immunol. 26, 1105–1112.

[10] Bertho, J.M., Fourcade, C., Dalloul, A.H., Debré, P. and Mossalayi, M.D. (1991) Eur. J. Immunol. 21, 1073–1076.

[11] Liu, Y.J., Cairns, J.A., Holder, M.J., Abbot, S.D., Jansen, K.U., Bonnefoy, J.Y., Gordon, J. and Maclennan I.C.M. (1991) Eur. J. Immunol. 21, 1107–1114.

[12] Aubry, J.P., Pochon, S., Graber, P., Jansen, K.U. and Bonnefoy, J.Y. (1992) Nature 358, 505–507.

[13] Suter, U., Bastos, R. and Hofstetter, H. (1987) Nucleic Acids Res. 15, 7295–7308.

[14] Towbin, H., Staehelim, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 4350–4354.

[15] Nunez, R. and Lynch, R.G. (1993) Pathobiology 199, (in press).

[16] Gower, H.J., Barton, C.H., Elsom, V.L., Thompson, J.T., Moore, S.E., Dickson, G. and Walsh, F.S. (1988) Cell 55, 955–964.

[17] Baumbach, W.R., Horner, D.L. and Logan, J.S. (1989) Genes and Development 3, 1199–1205.

[18] Mosley, B., Beckmann, M.P., March, C.J., Idzerda, R.L., Gimpel, S.D., VandenBos, T., Friend, D., Alpert, A., Anderson, D., Jackson, J., Wignall, J.M., Smith, C., Gallis, B., Sims, J.E., Urdal, D., Widmer, M.B., Cosman, D. and Linda, S.P. (1989) Cell 59, 335–348.

[19] Werner, S., Duan, D.R., Vries, C., Peters, K.G., Johnson, D.E. and Williams, L.T. (1992) Mol. Cell. Biol. 12, 82–88.

[20] Neefjes, J.J. and Ploegh, H.L. (1992) Immunol. Today 13, 179–184.