Biochemical and Biophysical Research Communications **287,** 35–41 (2001)
doi:10.1006/bbrc.2001.5539, available online at http://www.idealibrary.com on IDEAL®

DIgR1, a Novel Membrane Receptor of the Immunoglobulin Gene Superfamily, Is Preferentially Expressed by Antigen-Presenting Cells

Kun Luo,* Weiping Zhang,† Lili Sui,† Nan Li,† Minghui Zhang,† Xianwei Ma,* Lihuang Zhang,* and Xuetao Cao*,†,[1]

**Institute of Immunology, Zhejiang University, 353 Yanan Road, Hangzhou, 310031, People's Republic of China; and †Institute of Immunology, Second Military Medical University, 800 Xiangyin Road, Shanghai 200433, People's Republic of China*

Received August 6, 2001

A novel membrane receptor of immunoglobulin gene superfamily (IgSF) has been identified from mouse dendritic cells (DC) and designated as DC-derived Ig-like receptor 1 (DIgR1). It encodes a 228-amino-acid (aa) residue polypeptide with a 21-aa signal peptide, a 20-aa transmembrane region, a 189-aa extracellular region, and a 19 aa intracellular region. Its extracellular region contains a single V domain of Ig. So it is a novel type I transmembrane glycoprotein of IgSF. DIgR1 shows significant homologies to human CMRF-35 antigens and polymeric immunoglobulin receptors (pIgR). The mRNA expression of DIgR1 was highly abundant in mouse spleen. The preferential expression of DIgR1 mRNA is observed in the known antigen-presenting cells (APC) including DC, monocytes/macrophages, and B lymphocytes. A 40 kDa of protein in NIH/3T3 cells transfected with the DIgR1 cDNA was detected by Western blot analysis using anti-DIgR1 polyclonal antibodies. The expression of DIgR1 protein on DC is not regulated by LPS stimulation. Further study should be conducted to investigate what were biological functions of DIgR1 in the immunobiology of APC.

Key Words: **membrane receptor; immunoglobulin gene superfamily; antigen-presenting cell; dendritic cell; gene cloning.**

The immunoglobulin gene superfamily (IgSF) is a group of cell surface glycoproteins with similar structure. The typical IgSF member shares three conserved regions which mediated protein–protein interaction. In the N-terminus, the extracellular region generally contains one or more Ig-like domains consisting of 90–110 amino acids each and is putatively involved in molecular recognition. The transmembrane region composes of approximately 20 hydrophobic amino acids anchoring the IgSF molecule in cell membrane surface. The cytoplasmic region at the C-terminus with great variant contributes to the function of signal transduction. These molecules locate on the surface of leukocytes, epithelial cells, and nervous cells and make their functions on molecular recognition, cell activation, and cell–cell interaction (1, 2). The known members of IgSF including the major histocompatibility complex (MHC) class I and class II molecules, the T cell receptors, and T cell accessory molecules CD4 and CD8 (3), certain adhesion molecules (4, 5), cytokine receptors (6), and the polymeric immunoglobulin receptors (pIgR) (7, 8).

In recent years, a number of IgSF members including the CMRF-35 antigens have been identified (9–11). The CMRF-35 monoclonal antibody (mAb) produced by immunization with large granular lymphocytes (LGL) recognizes an epitope that presents on the surface of monocytes, neutrophils, a proportion of peripheral blood T and B lymphocytes and lymphocytic cell lines. The recognized epitope was found on at least two cell surface molecules, the CMRF-35A (12, 13) and CMRF-35H (14). These two CMRF-35 molecules share a highly homologus V-Ig domain (>90%) in the extracellular region, but their intracellular regions are quite different. CMRF-35H contains three putative immunoreceptor tyrosine-based inhibitory motifs (ITIM) in the cytoplasmic domain, suggesting that this molecule might play a negative regulatory role in leukocyte function. Meanwhile, CMRF-35A molecule has a short cytoplasmic domain without ITIM or other putative signaling motifs and a glutamic acid residue in its transmembrane domain, suggesting its potential association with an as yet undefined signaling molecule. They are expressed independently of each other on leukocyte populations and on hematopoietic cell lines and are the products of different genes (14, 15). These

[1] To whom correspondence should be addressed. Fax: (+86-21) 6538 2502. E-mail: caoxt@public3.sta.net.cn.

0006-291X/01 $35.00

suggest that these two molecules may play distinct but related roles in the regulation of leukocyte function.

The function of these molecules remains unknown, however they have been implicated to be associated with cell activation/differentiation, molecular recognition, and regulation. Here, we report a novel member of IgSF isolated from mouse dendritic cells (DC) which shares high homology to CMRF-35A. We designated it as dendritic cell-derived Ig-like receptor 1 (DIgR1). DIgR1 is a type I transmembrane glycoprotein which is highly expressed in spleen and is preferentially expressed in the professional antigen-presenting cells (APC) including DC, monocytes/macrophages, and B lymphocytes.

MATERIALS AND METHODS

Cell preparation. Mouse DC culture procedure used in this study was described previously (16). Briefly, bone marrow cells from BALB/c mice were depleted of red blood cells with ammonium chloride and depleted of T, B cells and Ia^+ cells using a cocktail containing anti-CD4, anti-CD8, anti-B220/CD45R, and anti-Ia monoclonal antibodies (ATCC). Then, the cells were plated in 24-well culture plates (10^6 cells/well) in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 50 μM 2-ME, 10 mM Hepes (pH 7.4), 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 3.3 ng/ml recombinant murine GM-CSF (Sigma), and 1 ng/ml recombinant murine IL-4 (Sigma). At day 3 of culture, floating cells were gently removed and adherent cells were cultured in fresh medium. At day 9, nonadherent and loosely adherent cells were harvested and used as DC population with the purity of >90%. For activation, DC were treated with LPS (2 μg/ml) for 24 h. Freshly isolated T and B lymphocytes from BALB/c mice were prepared using positive selection with anti-CD8, anti-CD4, or anti-B220/CD45R monoclonal antibody (ATCC) and Minimagnetic bead-conjugated secondary antibody (Militenyi Biotec Ginbit). The labeled cells were enriched by passing through a MiniMACS column placed in a magnetic field (MiniMacs, Militeny Biotec, Bergisch, Germany).

Isolation of DIgR1 full-length cDNA. By BLAST analysis of mouse EST databases from the National Center for Biotechnology Information, three mouse ESTs (GenBank Accession Nos. AI645367, AI060397, and AA175302) were found to be highly homologous to human CMRF-35 antigens, from which mouse DIgR1 full-length cDNA was obtained by contig. According to this available sequence, RT-PCR was performed to clone DIgR1 cDNA from mouse DC. The upstream primer of DIgR1 is 5′-GGAATTCACCAGGAAGCAGAGGA-3′, and the downstream primer is 5′-CGGGATCCTCAGCCACCAGAGGAGCTGT-3′. The amplification parameters were denaturing of 30 s at 94°C, annealing of 30 s at 57°C, and extension of 45 s at 72°C on a thermocycle PCR9600 (Applied Biosystem Inc.). After 30 cycles of amplification the PCR product was digested by *Eco*RI/*Bam*HI and inserted into pGEM-3Z(f) vector (Promega, Madison, WI) and the sequence was conformed by DNA sequencing.

Northern blotting. Northern blotting was performed as described previously (17). Briefly, DIgR1 cDNA insert excised by enzymatic digestion and ^{32}P labeled was used as a probe. Ready-to-use blots containing mouse RNA from various tissues (2 μg/lane) were purchased from Clontech Laboratories. The filters were hybridized with ^{32}P-labeled cDNA probes in ExpressHyb hybridization solution (Clontech Laboratories) according to the manufacturer's instructions. After stringently washing at 50°C for 20 min in 0.1 × SSC and 0.1% SDS, the filters were subjected to autoradiography. The filters were stripped and reprobed with mouse β-actin cDNA probe (Clontech Laboratories).

RT-PCR analysis of DIgR1 expression. Murine cell lines used for analysis of mRNA expression and Western blotting were listed here: NIH/3T3 (embryo fibroblast cell), P815 (mastocytoma), Raw (macrophage; monocyte), B16 (melanoma), YAC-1 (lymphoma), A20 (B lymphocyte), EL4 (T lymphocyte), CTLL-2 (T lymphocyte), E.G7 (lymphoma; T lymphocyte), FBL3 (erythroleukemia), WEHI164 (fibrosarcoma), CT26 (colonocarcinoma). All of these cell lines were obtained from ATCC. Standard procedures were used for cell culture. Total cellular RNA was isolated using Trizol reagent (Life Technologies) and then was reverse-transcribed into cDNA, The PCR was performed with the following primer sets: 5′-GGAATTCACCAGGAAGCAGAGGA-3′ and 5′-CGGGATCCTCAGCCACCAGAGGAGCTGT-3′ for DIgR1; and 5′-TGGAATCCTGTGGCATCCATGAAAC-3′ and 5′-TAAAACGCAGTAACAGTCCG-3′ for β-actin.

Recombinant expression of GST fusion protein of DIgR1 and generation of anti-DIgR1 polyclonal antibodies. The cDNA encoding DIgR1 extracellular region without signal peptide was amplified by PCR with the sense primer 5′-GGATCCTCTCAGGTCCCAGGCTGT-3′ and antisense primer 5′-GAATTCAGGGAGCAGGAATTGTGTCATC-3′. The DIgR1 cDNA sequence and the ORF were confirmed by DNA sequencing, then inserted into the expression vector pGEX-2T (Pharmacia Biotech, Piscataway, NJ). The *Escherichia coli* strain BL21 was used as host to express GST fusion protein. The soluble fusion protein was obtained under the condition of IPTG (0.5 mM) induction at 22°C for 3 h and purified by affinity chromatography (Pharmacia Biotech). Female New Zealand rabbits were immunized with purified fusion protein to produce polyclonal antibodies according to conventional procedure. DIgR1 fusion protein was injected into multiple subcutaneous sites and immunizations were boosted twice at 3-week intervals. Ten days after the last injection, the anti-DIgR1 serum was isolated and purified using protein A affinity chromatography (Pierce, Rockford, IL). The titration of this antiserum was detected by Western blot.

Eukaryotic expression of recombinant DIgR1 protein. To express His-tagged DIgR1 protein in eukaryotic cells, DIgR1 cDNA was amplified by PCR and inserted into vector pcDNA3.1/mic-His (−) (Invitrogen). The DNA sequence was confirmed by DNA sequencing. The transcription was initiated by CMV promoter. NIH/3T3 cells were transfected with DIgR1 expression vector using the Lipofactamine transfection reagent (Gibco) according to the manufacturer' s instructions. After 3 weeks of screening in the presence of 800 μg/ml G418 (Calbiochem, La Jolla, CA), the stable positive clones were obtained and confirmed by Western blot analysis using anti-DIgR1 polyclonal antibodies. These stable expression clones were used for immunoblotting.

Immunoblotting analysis. Totally harvested cells were lyzed in cell lysis buffer (1% NP-40, 50 mM Tris–CL, pH 7.8, 150 mM NaCl) containing PMSF (100 μg/ml), aprotinin(1 μg/ml), and leupeptins(1 μg/ml). The lysates were fractionated by 12% SDS–PAGE gel and transferred onto nitrocellulose membrane. After blocked with 10% nonfat dry milk for 2 h, the membrane was washed three times for 5 min each with TBST (100 mM Tris–Cl, pH 7.6, 0.9% NaCl, 0.1% Tween 20). Then the membrane was incubated in anti-DIgR1 polyclonal antibodies (1:2000 dilution) over night at 4°C. After washed three times for 5 min each with TBST, the membrane was incubated in HRP-coupled anti-rabbit secondary antibody (New England Biolabs Inc., 1:20000 dilution) for 1 h. After washed with TBST as above, the membrane was incubated in LumiGLo for 1 min and then wrapped in Saran Wrap to expose to X-ray film.

FACS analysis. Cells growing vigorously were harvested, then were washed twice with PBS, and incubated in anti-DIgR1 polyclonal antibodies for 1 h at 4°C. Preimmune rabbit serum were used as negative control. After being washed three times for 5 min each, the cells were incubated in FITC-conjugated anti-rabbit IgG (DAKO) for 30 min. All cells were analyzed by cytofluograph on a FACScan (Becton–Dickinson, San Jose, CA) equipped with lysis software).

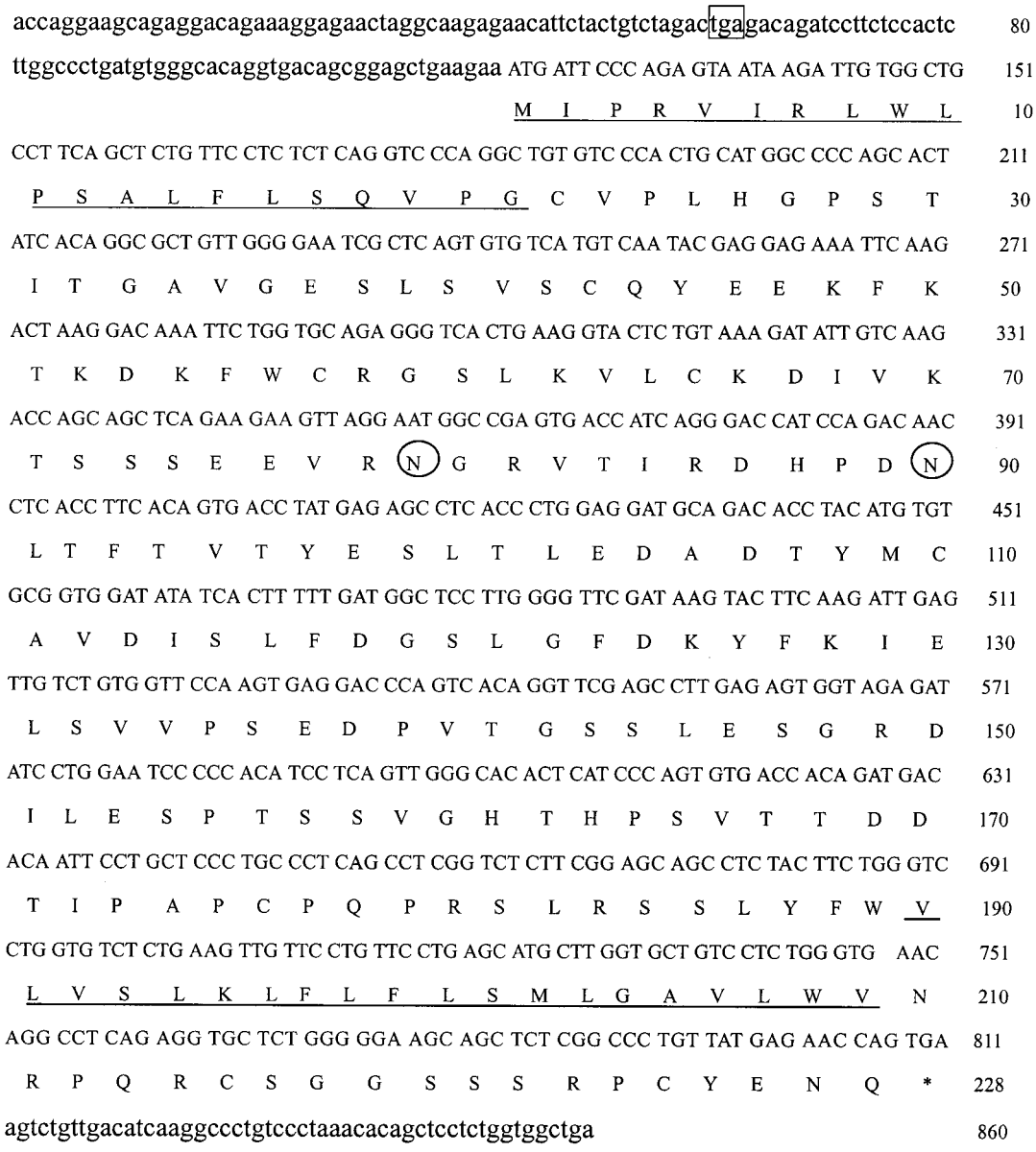

FIG. 1. Sequences of nucleotides and deduced amino acids of DIgR1. The in-frame stop codons in 5′ UTR are boxed. Single underline is the signal peptide. Double underline is the transmembrane domain. The potential N-glycosylation sites are cycled. The sequence of DIgR1 has been deposited in the GenBank/EMBL under the Accession No. AY048685.

RESULTS

Identification and Sequence Analysis of DIgR1

By searching mouse EST database against human CMRF-35 antigens, we obtained three ESTs (GenBank Accession Nos. AI645367, AI060397, and AA175302) which were contiged to form a full-length cDNA with a complete open-reading frame (ORF) potentially encoding a peptide homologous to human CMRF-35A. By amplifying the cDNA with specific primers using RT-PCR from mouse bone marrow derived DC, we identified the full-length cDNA. The 860-bp full-length cDNA contains ORF of 684 bp and a 5′ untranslating region (UTR) of 121 bp containing a stop codon TGA. It indicated that it was a full-length cDNA clone. The mature DIgR1 protein has a length of 228 aa with four conserved cysteine residues and two potential N-linked glycosylation sites, with the predicted molecular mass of 25.2 kDa before glycosylation. A putative hydrophobic signal anchor sequence of 20 aa extending from nt 689 to 748 was identified as an integral transmembrane region. The 189-aa extracellular region met the structural features of Ig-V domain and is predicted to carry 21-aa signal polypeptide in its NH2-terminus (Fig. 1). So it belongs to type-I transmembrane receptor of IgSF. The novel molecule is designated as DIgR1.

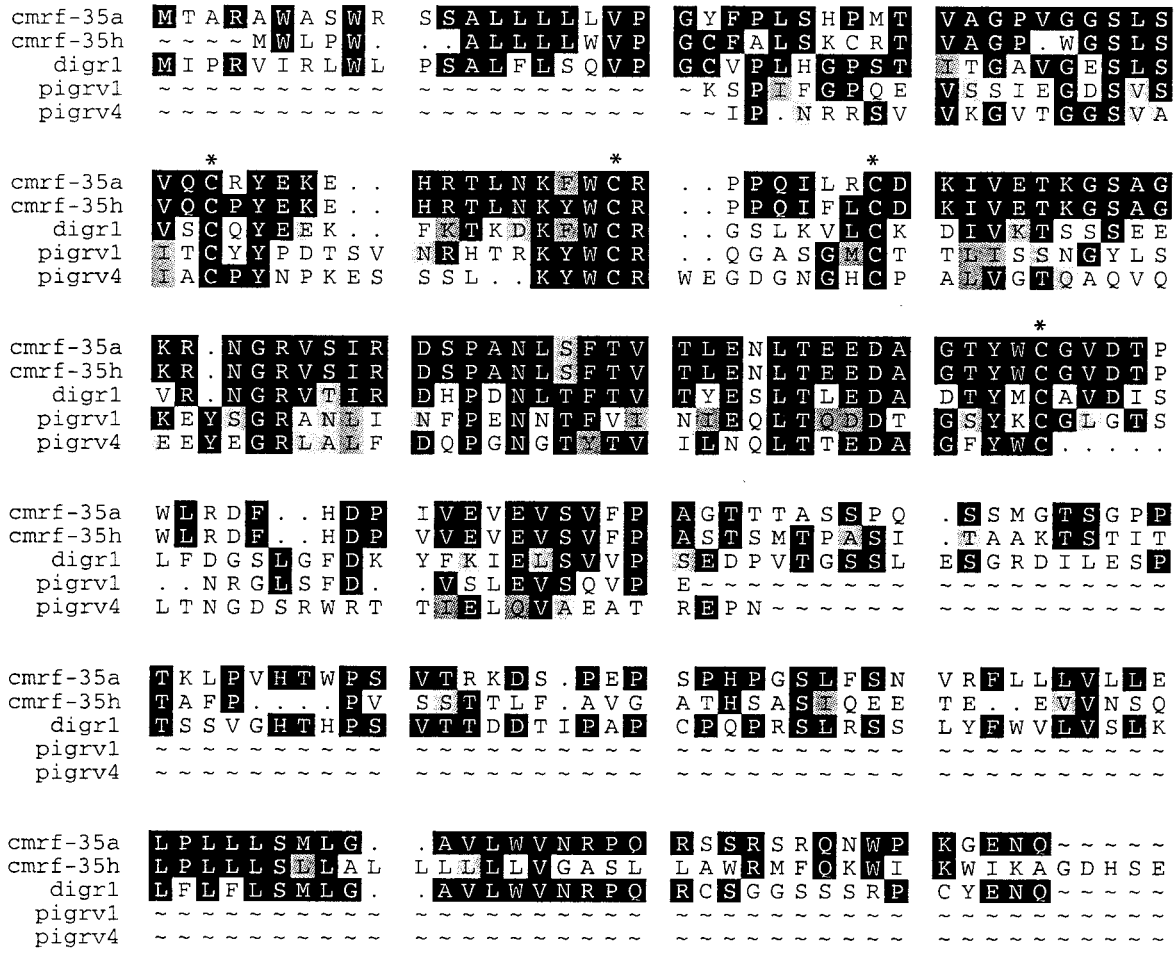

FIG. 2. Multiple alignment of the deduced DIgR1 protein with human CMRF-35A, CMRF-35H, V1, and V4 domains of mouse pIgR. Identical amino acids are shown on black background. Similar residues are in gray. The conserved Cys residues in extracellular region are marked with asterisks.

Identity of amino acid sequence (45%) between DIgR1 and human CMRF-35A was revealed (Fig. 2). The extracellular region of DIgR1 was also significantly homologous to human CMRF-35H (53% identity), V1 domain of mouse pIgR (30% identity), and V4 domain of mouse pIgR (36% identity). Similar homology was founded between DIgR1 and rabbit or rat pIgR (not shown).

DIgR1 mRNA Expression Patterns in Mouse Dendritic Cells, Monocytes/Macrophages, and B Cells

Northern blotting revealed approximately 0.9 and 2.0 kb transcripts in several normal mouse tissues (Fig. 3). The greatest expression of DIgR1 mRNA was observed in the spleen and the 0.9 kb transcript was dominant. These two different transcripts might be generated by alternative splicing. Faint expression was detected in heart, spleen, lung, liver, and testis. No expression was observed in kidney, brain, and skeletal

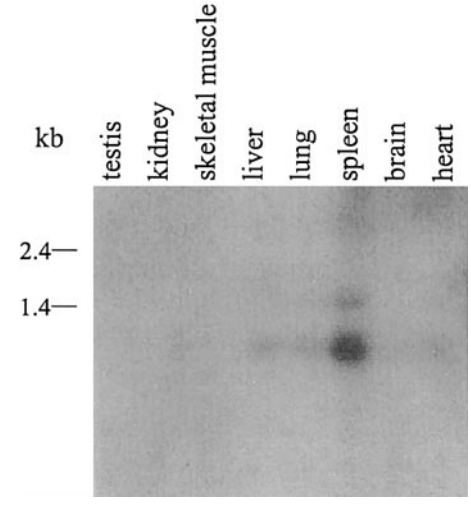

FIG. 3. Northern blot analysis of DIgR1 expression in normal tissues. Poly(A)+ RNA from the indicated tissues were hybridized with radioactive probes which contain the full-length sequence of DIgR1. The mRNA markers are shown in size on the left.

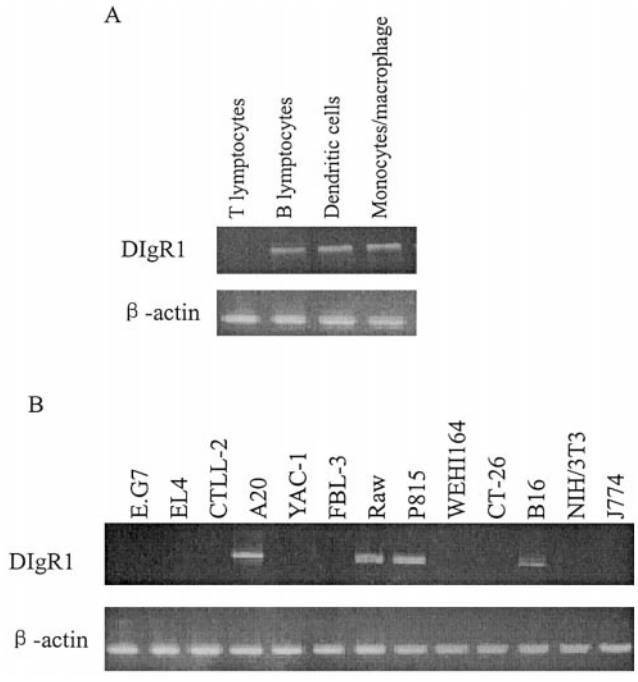

FIG. 4. RT-PCR analysis of DIgR1 mRNA expression. Mouse β-actin was amplified as positive control. (A) RT-PCR products of freshly isolated mouse T lymphocytes, B lymphocytes, monocytes, and bone marrow-derived DC. (B) RT-PCR products of partly hematopoietic and tumor cell lines.

muscle. Highly abundant expression of DIgR1 mRNA in spleen indicated that it might possess important function in the immune system. We further investigated its cellar distribution by RT-PCR analysis in various cells or cell lines (Fig. 4). In hematopoietic cells and cell lines, expression of DIgR1 mRNA was observed in freshly isolated murine bone marrow-derived DC, monocytes, B lymphocytes, and B lymphoma cell line (A20), monocytes/macrophage cell line (Raw), but no expression was detected in either freshly isolated T lymphocytes or T cell lines (EL4, CTLL-2, or E.G7). DIgR1 mRNA was expressed in mouse mature DC with high abundance. Different levels of DIgR1 expression were also seen in some tumor cell lines. Therefore, DIgR1 was expression preferentially by DC, monocytes, and B lymphocytes. As we know, DC, monocytes, and B lymphocytes are professional APC which play important roles in the initiation and regulation of immune response. So DIgR1 may be related to the immunobiology of APC.

Expression of DIgR1 Protein in DIgR1-Transfected Cells

To further investigate the biological characterization of DIgR1, we generated GST-DIgR1 fusion protein and produced anti-DIgR1 serum. The purified polyclonal antibodies could react with 0.05 μg of DIgR1 protein at the titer of 1:4000 and no cross-reactivity with bacterial protein. Then the DIgR1 cDNA with full-length encoding region was inserted into pcDNA3.1 vector that was transfected into NIH/3T3 cells for stable expression of DIgR1 protein. A ~40 kDa protein was detected in transfected NIH/3T3 cells under reduced and non-reduced condition by Western-blotting with rabbit polyclonal antibodies against DIgR1 (Fig. 5). This indicates the mature protein of DIgR1 is a monomer and is highly glycosylated.

Expression of DIgR1 Protein on the Surface of Mouse Dendritic Cells

DC are the most potent professional APC in the immune system, and the one capable of sensitizing naive, unprimed T cells. Importantly, DC express high level of MHC and costimulatory/adherent molecules which are necessary to activate T cells. Because DIgR1 mRNA was expressed in mouse DC with high abundance, we want to know what level of DIgR1 protein expression on DC at different stages in order to further study the role of DIgR1 in immunobiology of DC. DIgR1 on mouse DC was detected by FACS. DCs from mouse bone marrow were cultured in media containing GM-CSF and IL-4 for 7 days DC, 9 days DC, or activated with LPS for 24-h activated DC. FACS analysis revealed high expression of DIgR1 protein on DCs (Fig. 6A). But no significant difference in DIgR1 expression was observed among 7 day DC, 9 day DC, and activated DC. This phenomenon was also confirmed by RT-PCR and Western blotting analysis (Figs. 6B and Fig. 6C).

DISCUSSION

In this study, we isolated a novel cell surface glycoprotein named DIgR1 from mouse DC. Characterized by consisting of a single V-Ig domain in its N-terminus, this 228-residue protein belongs to IgSF. DIgR1 shares the closest homology to CMRF-35A, which putatively has an important function common to diverse leukocyte types. Just like human CMRF-35A, DIgR1 con-

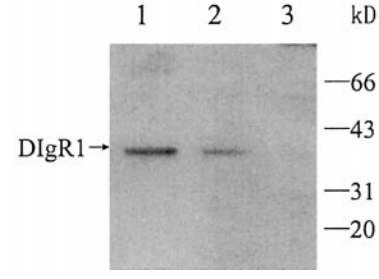

FIG. 5. Western blot analysis of DIgR1 protein expression in DIgR1 cDNA transfected NIH/3T3 cells under reduced (lane 1) and nonreduced (lane 2) conditions. NIH/3T3 cells transfected pcDNA3.1 was subjected to negative control (lane 3). Protein markers are shown on the right.

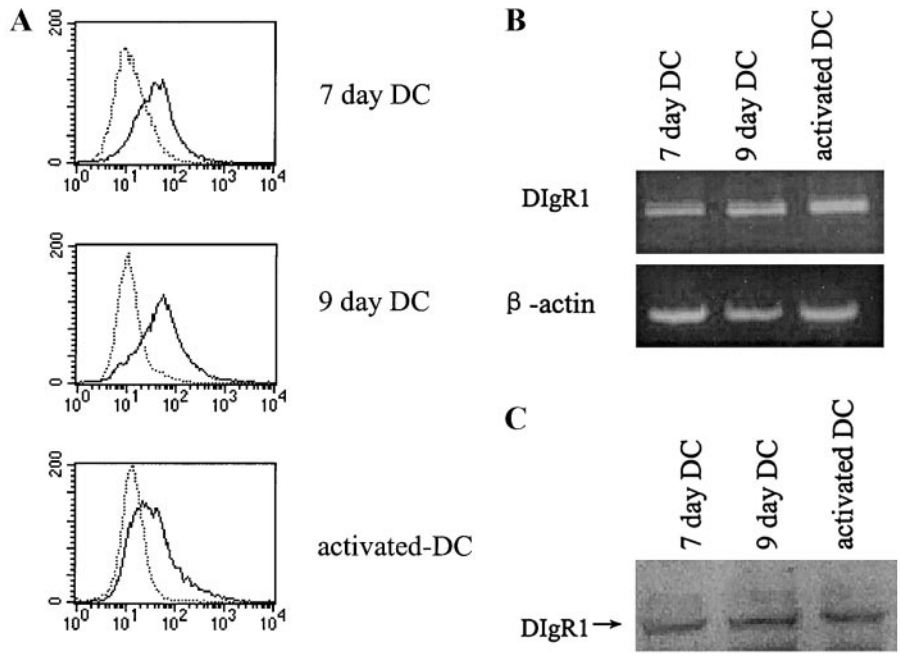

FIG. 6. Analysis of DIgR1 expression on 7 day DC, 9 day DC, and LPS-activated DC. (A) Expression analysis of DIgR1 protein by FACS. Solid lines show that the cells were stained with anti-DIgR1 polyclonal antibodies. Dotted lines show the cells were stained with preimmune sera as negative control. No difference in DIgR1 protein expression was observed on DC at different stages that were consistent with the results of RT-PCR (B) and Western blot analysis (C).

tains a short cytoplasmic domain indicating that this molecule might be associated with an as yet undefined signaling molecule to make functions. Within the extracellular region, DIgR1 shares sequence homology with CMRF-35H (14, 15), but DIgR1 lacks immunoreceptor tyrosine-based inhibitory motif (ITIM) that negatively regulates the activation of cells when cocrosslinked with immunoreceptor tyrosine-based activatory motif (ITAM) in its cytoplasmic domain (18, 19). So DIgR1 might be a noninhibitory (or activated) molecule on the surface of leukocytes. In contrast to expression pattern of CMRF-35A, DIgR1 mRNA is not expressed in T lymphocytes on which CMRF-35A transcripts are positive when confirmed by RT-PCR. Therefore, DIgR1 might have distinct functions when compared to CMRF-35A.

On the other hand, DIgR1 Ig-like domain is most closely related to the V1 and V4 domain of mouse pIgR. The pIgR, which is constitutively expressed on the basolateral surface of secretory epithelial cells, mediates external translocation of polymeric IgA, and pentameric IgM (collectively called pIg) to exocrine secretions (20, 21). Studies on the structure and function of pIgR reveal that there are six Ig-like domains in the extracellular pIg-binding region of the receptor (22). But each domain is not required for IgA binding. Domain I has high affinity for binding to IgA (23). The deletion of domains II and III or individual deletion of domains IV and V of pIgR does not prevent binding to IgA (24, 25). But no study demonstrates that the extracellular region of pIgR harboring a single domain I can bind to IgA. It is interesting that the expression of DIgR1 mRNA is highly abundant in small intestine and duodenum, which is revealed by RT-PCR analysis (data not shown). These tissues highly expressing pIgR at a high level are vigorous in mucosal immune response. Our further investigation will focus on the binding activity of DIgR1 with IgA.

Northern blotting analysis indicating high expression of DIgR1 in the spleen showed that DIgR1 might possess important function in the immune system. Then, its cellar distribution was analyzed by RT-PCR in various cell lines and freshly isolated cells. High level expression of DIgR1 mRNA was detected in the unactivated DC and activated DC. In hematopoietic cell lines, expression of DIgR1 mRNA was observed in B lymphocytes and B lymphoma cell line (A20), macrophages and cell line (Raw), but no expression was detected in T lymphocytes and T cell lines (EL4, CTLL-2, E.G7). DC, monocytes/macrophages, and B lymphocytes are all of professional APCs. So DIgR1 is speculated to be involved in the immunobiology of APC such as antigen capture, process and presentation.

In conclusion, we identify a novel cell membrane receptor DIgR1 that belongs to IgSF. DIgR1 is highly expressed in spleen and is preferentially expressed in professional APC including DC, monocytes/macrophages, and B lymphocytes, suggesting that DIgR1 might play roles in the immune system. But the expression of DIgR1 on DC can not be regulated by

dangerous signals such as LPS stimulation. What are the factors that can regulate the expression of DIgR1 in the immune system? What are the functions of DIgR1 in the immunobiology of APC? These need to be characterized by further investigation.

ACKNOWLEDGMENTS

We sincerely thank Mrs. Yan Li, Mei Jin, Chunfang Luo, Mr. Daoming Zhang, and Guohua Lu for their excellent technical assistance.

REFERENCES

1. Williams, A. F., and Barclay, A. N. (1988) *Annu. Rev. Immunol.* **6,** 381–405.
2. Hunkapiller, T., and Hood, L. (1989) *Adv. Immunol.* **44,** 1–63.
3. Littman, D. R. (1987) *Annu. Rev. Immunol.* **5,** 561–584.
4. Starling, G. C., Egner, W., Mclellan, A. D., Fawcett, J., Simmons, D. L., Hart, D. N. J. (1995) *Eur. J. Immunol.* **25,** 2528–2532.
5. Hart, D. N., and Prickett, T. C. (1993) *Cell Immunol.* **148,** 447–454
6. Halaby, D. M., and Mornon, J. P. (1998) *J. Mol. Evol.* **46,** 389–400.
7. Kushiro, A., and Sato, T. (1997) *Gene* **204,** 277–282.
8. Piskurich, J. O., Blanchard, M. H., Youngman, K. R., France, J. A., and Kaetzel, C. S. (1995) *J. Immunol.* **154,** 1735–1747.
9. Selvakumar, A., Steffens, U., and Dupont, B. (1996) *Tissue Antigens* **48,** 285–295.
10. Cella, M., Dohing, C., Samaridis, J., Dessing, M., Brockhaus, M., Lanzavecchia, A., and Colonna, M. (1997) *J. Exp. Med.* **185,** 1743–1751.
11. Fournier, N., Chalus, L., Durand, I., Garcia, E., Pin, J., Abrams, J., E. M. Bates. E., and Garrone, P. (2000) *J. Immunol.* **165,** 1197–1209.
12. Jackson, D. G., Hart, D. N., Starling, G., and Bell, J. I. (1992) *Eur. J. Immunol* **22,** 1157–1163.
13. Daish, A., Starling, G. C., Mckenzie, J. L., Nimmo, J. C., Jackson, D. G., and Hart, D. N. (1993) *Immunology* **79,** 55–63.
14. Green, B. J., Clark, G. J., and Hart, D. N. (1998) *Int. Immunol.* **10**(7), 891–899.
15. Clark, G. J, Green, B. J., and Hart, D. N. (2000) *Tissue Antigens* **55**(2), 101–109.
16. Cao, X., Zhang, W., He, L., Xie, Z., Ma, S., Tao, Q., Yu, Y., Hamada, H, and Wang, J. (1998) *J. Immunol.* **161,** 6238–6244.
17. Cao, X., Zhang, W., Wan, T., He, L., Chen, T., Yuan, Z., Ma, S., Yu, Y., and Chen, G. (2000) *J. Immunol.* 2588–2595.
18. Bolland, S., and Ravetch, J. (1999) *Adv. Immunol.* **72,** 149–177.
19. Gergely, J., Pecht, I., and Sarmay, G. (1999) *Immunol. Lett.* **68,** 3–15.
20. Kaetzel, C. S., Robinson, J. K., Chintalacharuvu, K. R., Vaerman, J. P., and Lamm, E. M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8796–8800.
21. Kaetzel, C. S., Robinson, J. K., and Lamm, E. M. (1994) *J. Immunol.* **152,** 72–76.
22. Piskurich, J. F., Blanchard, M. H., Youngman, K. R., France, J. A., and Kaetel, C. S. (1995) *J. Immunol.* **154,** 1735–1747.
23. Bakos, M. A., Kurosky, A., Woodard, C. S., and Denney, R. M. (1991) *J. Immunol.* **146,** 162–168.
24. Coyne, R. S., Siebrecht, M., Peitsch, M. C., and Casnnovr, J. E. (1994) *J. Biol. Chem.* **269,** 31620–31625.
25. Crottet, P. C., Peitsch, M. C., Servis, C., and Corthesy, B. (1999) *J. Biol. Chem.* **274,** 31445–3145.