Molecular cloning of murine decay accelerating factor by immunoscreening

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Abstract

Although the cDNA of human decay accelerating factor (DAF) which restricts complement activation on homologous cell membranes was cloned in 1987, all trials to detect the cDNA of mouse DAF by cross-hybridization were unsuccessful. However, by immunoscreening with a rabbit antiserum against purified mouse DAF, we successfully cloned the cDNA. It contains four typical short consensus repeats (SCR) similar to that in human and guinea pig DAF. The base sequence showed 63.7 and 63.8% identity to that of human and guinea pig DAF respectively. The deduced amino acid sequence identity to human and guinea pig DAF was 47.2 and 46.5% respectively. Mouse complement receptor related gene Y (Crry)/p65 function is comparable to DAF. SCR3 and SCR4 of mouse DAF showed 50% identity to SCR2 and SCR3 of Crry/p65 respectively.

Identification of the mouse DAF gene should open a new approach for determining the actual in vivo role of DAF by analyzing autoimmune mice as well as generating DAF gene knockout mice using embryonic stem cells.

Introduction

Several membrane molecules which restrict the autologous complement reaction have been demonstrated on human cells. Those are decay accelerating factor (DAF) (1-3), membrane cofactor protein (MCP) (4,5) and 20 kDa homologous restriction factor (HRF20) (6). HRF20 is also known as CD59 (7), MAC inhibitory factor (8,9) and membrane inhibitor of reactive lysis (10) due to simultaneous discovery in different laboratories. These membrane inhibitors play a role in protecting self cells from the action of autologous complement which is self activating because C3 molecules continuously, although slowly, automatically convert to the active form (11,12) due to hydrolysis of a thioester bond in the α-chain by a water molecule which happens to penetrate the molecule (13). DAF and MCP restrict the amplification reaction at C3 convertase and HRF20 (CD59) restricts the terminal step of complement resulting in membrane damage.

Previously, we purified mouse DAF (14) from mouse erythrocytes in a series of chromatographic steps using its inhibitory function on C3 convertase to monitor its activity. Then, antiserum against mouse DAF (anti-mouse DAF) was prepared by immunizing a rabbit with the purified antigen. It is important to analyze DAF function in the mouse because (i) the role of DAF in various diseases can be examined in the mouse model of human disease and (ii) previously unknown functions of DAF in vivo can be examined using transgenic and gene targeting methods in the mouse. Many attempts to clone mouse DAF cDNA by PCR were carried out as well as cross-hybridization methods using homology to human DAF without success. Since there is another molecule, complement receptor related gene Y (Crry)/p65, in the mouse with DAF activity, it was argued that the mouse counterpart of human DAF may not exist, although we previously reported biochemical purification of mouse DAF (14). Now we demonstrated the presence of DAF in the mouse complement system by cloning mouse DAF cDNA using immunoscreening. We examined its homology with human and guinea pig DAF (3,15), and also with mouse Crry/p65 (16-18).

Methods

Materials

[α-32P]dCTP was purchased from Amersham Japan (Tokyo, Japan). A mouse spleen cDNA library constructed in a Lambda ZAPII vector was purchased from Clontech (Palo Alto, CA). Rabbit anti-mouse DAF antibody was prepared as
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**Fig. 1** Nucleotide and deduced amino acid sequence of mouse DAF. The glycosylation signal is indicated by double underlining. Nucleotide positions of primers for PCR amplification are underlined. The sequence data are available from DDBJ/EMBL/Genbank under accession number DQ091234.
Immunoscreening of murine DAF cDNA

Fig. 2. Nucleotide and deduced amino acid sequences of mouse DAF isolated from a mouse lung cDNA library. The polyadenylation signal is indicated by double underlining as in Fig. 1. Several nucleotides and amino acids are different from those of mouse DAF cDNA obtained by immunoscreening.
Immunoscreening of murine DAF cDNA

The mouse DAF cDNA isolated by immunoscreening was amplified by PCR from the signal peptide protein to the C-terminal portion using the 5'-GCACGAGCTGGCCATCAC-3' (sense strand) and 5'-CTATGTCAAGTAGCCAAT-3' (anti-sense strand). Amplified cDNA was 32P-labeled using a random primer labeling kit (Primer-IT II; Stratagene, La Jolla, CA) and hybridized to a mouse lung cDNA Xgt11 library (Clonetech) at 60°C for 16 h in a buffer containing 5xSSC, 0.02% SDS, 0.1% Sarkosy (Fluka, Ronkonkonk, NY), 1% blocking reagent in DIG detection kit (Boehringer Mannheim, Indianapolis, IN) and 0.1 mg/ml salmon sperm DNA (Wako, Osaka, Japan). Positive clones were purified and the inserted DNAs were subcloned into pBluescript (Stratagene) and sequenced.

Northern blotting

PCR amplification of SCR2 to the C-terminal portion of mouse DAF cDNA was carried out using 5'-AAATCATGTGTT-GCTCCAGAAAGACTGAGT-3' (sense strand) and 5'-CTA-TGTCAAGTAGCCCAAT-3' (anti-sense strand). The PCR fragment was labeled with [a-32P]dCTP by the random priming. Mouse total RNA was isolated using Isogen (Nippon Gene, Tokyo, Japan) and electrophoresed in a 1% agarose gel, and transferred to a Hybond N+ nylon membrane (Amersham...
Immunoscreening of marine DAF cDNA

Fig. 4. Comparison of the amino acid sequences of SCR regions of mouse DAF and Crry/p65.

Fig. 6. RT-PCR analysis of various mouse tissues. The region from SCR4 to the 3' end as shown in Fig. 1 was amplified. Each first cDNA strand was derived from brain (lane 1), kidney (lane 2), lung (lane 3), liver (lane 4) and spleen (lane 5). DNA size makers are on the right.

Results and discussion

A cDNA which has a homology to human and guinea pig DAF was successfully cloned using anti-mouse DAF for immunoscreening 6 x 10^5 clones infected with Lambda ZAP II phage containing a C57BL/6 mouse spleen cDNA library. We then termed the clone tentatively mouse DAF cDNA. The nucleotide and deduced amino acid sequences are shown in Fig. 1. The cDNA encodes a protein of 356 amino acids with hydrophobic amino acid-rich signal peptides. An initiation codon is not apparent in this clone. We then isolated another mouse DAF cDNA clone from a mouse lung cDNA library using the PCR amplified portion of this clone. By screening a 5 x 10^5 mouse lung λgt11 library, 20 positive clones were obtained. Several clones were analyzed and found to have an initiation codon ATG as shown in Fig. 2. Several nucleotides and deduced amino acids of a mouse DAF clone derived from a lung library were different at the position indicated by arrows in Fig. 2 from that of mouse DAF isolated from a spleen library by immunoscreening. It is conceivable that it may be based on the difference in mouse lineage used to construct the cDNA library. The ZAP II cDNA library is derived from a BALB/c spleen and the λgt11 cDNA library is derived from C57BL/6 lung. A typical polyadenylation signal sequence (AATAAA) was found 80 bp downstream of the TAG sequence (Fig. 1) and with poly(A) tails (Fig. 2). The deduced amino acid sequence of mouse DAF cDNA was compared to the reported human (2,3) and guinea pig DAF (15). As shown in Fig. 3, mouse DAF shows 47.2 and 46.5%...
Immunoscreening of murine DAF cDNA

In the mouse system, a cDNA termed Crry has been cloned (16) by cross-hybridization with the cDNA of human complement receptor type 1 (CR1) and Crry/p65 has been demonstrated to restrict formation of C3 convertase (17,18). Since the function of Crry/p65 is essentially the same as that of DAF, we compared their amino acid sequences. As indicated in Fig. 4, relatively high homology in the SCR regions was observed. The homology of mouse DAF SCR2 to Crry SCR1 is 30%, that of mouse DAF SCR3 to Crry SCR2 is 50% and that of mouse DAF SCR4 to Crry SCR3 is 50%. From deletion experiments of each SCR region of human DAF, it was shown that SCR1 is not necessary for function, and that the SCR2 and SCR4 regions provide the proper conformation for the active site on SCR3 (19). Mouse H also has decay accelerating activity and the SCR 1–4 are reported to be functional sites (20). However, the amino acid identity of mouse DAF SCR with mouse H SCR is lower compared with Crry SCR. The identity of mouse DAF SCR2 with mouse H SCR2 is 23%, that of mouse DAF SCR3 with mouse H SCR3 is 27% and that of mouse DAF SCR4 with mouse H SCR4 is 26% respectively. These results mean that it is difficult to predict functional sites by comparison of amino acid sequences. However, the high homology between mouse DAF SCR3 and Crry SCR2 and between mouse DAF SCR4 and Crry SCR3 still suggest the presence of important sites in the conserved regions.

The PCR fragment from SCR2 to the 3′ end region of mouse DAF cDNA was labeled and used to probe a Northern blot. As shown in Fig. 5, hybridization revealed a strong signal at about 2.5 kb and a minor band of 1.8 kb. These message sizes are comparable with those of human DAF (3) and guinea pig DAF (15).

As reported by Nonaka et al. (15), guinea pig DAF has multiple serine/threonine-rich regions. Since such isoforms are not expressed in human DAF, we analyzed the possibility of the presence of the same types of isoforms in mouse DAF by RT-PCR. As shown in Fig. 6, only one predicted 500 bp band was detected in various tissues using primers from the SCR4 region to the 3′-end region. Even when primers from the SCR2 region to the 3′-end region were used, one predicted 880 bp band was detected (not shown). These results indicate that the multiple isoforms generated by alternative splicing of the serine/threonine-rich region are not expressed in the case of mouse DAF. Nonaka et al. (15) also reported that alternative splicing of two optional exons generates transmembrane and anchored forms of guinea pig DAF. The transmembrane form of mouse DAF was not identified in our screening of the mouse lung cDNA library.

Since various syngeneic mouse strains including those susceptible to several types of autoimmune diseases are available, identification of the mouse DAF gene should provide a way to investigate the actual in vivo role of DAF in development of autoimmune diseases. Furthermore, availability of embryonic stem cells in mice will also be an advantage in studies of the role of DAF in gene manipulated mice.

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Abbreviations

Crry complement receptor related gene y
DAF decay accelerating factor
HRF20 20 kDa homologous restriction factor
MCP membrane cofactor protein
SCR short consensus repeat

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