Identification of cDNA Encoding a Serine Protease Homologous to Human Complement C1r Precursor from Grafted Mouse Skin

Sung June Byun,*[1] Young Yil Bahk,*†[1] Zae Young Ryoo,†‡ Kyoung-Eun Kim,*† Ha-Young Hwang,* Jung-Woong Lee,‡ Jong-Yuk Yi,§ and Tae-Yoon Kim*

*Department of Dermatology-Immunology, Kangnam St. Mary Hospital, College of Medicine, Korea; ‡Research Institute of Animal Science, Catholic Research Institute of Medical Science, Korea; §Department of Dermatology, Uijongbu St. Mary Hospital, The Catholic University of Korea, Korea; †Bio Clue & Solution Co. Ltd., Korea

We isolated a cDNA clone from grafted mouse skin that encodes a serine protease homologous to human C1r. The C1r protease is involved in the activation of the first component of the classical pathway in the complement system. In order to identify novel transcripts whose expression is regulated in grafted mouse skin, we first perfomed differential display reverse transcription polymerase chain reaction analysis and obtained 18 partial cDNA clones whose protein products are likely to play an important role in allograft rejection. One of these showed significant sequence homology with human complement C1r precursor. The other clones displayed no homology to any known sequences, however. Northern blot analysis demonstrated that the level of this transcript was upregulated in day 8 postgrafted skin. The full-length cDNA 2121 nucleotides in length obtained from screening a mouse skin cDNA library contained a single open reading frame encoding 707 amino acid residues with a calculated molecular weight of 80,732 Da. Its deduced amino acid sequence revealed an 81% identity and 89% similarity to the human C1r counterpart. In particular, mouse C1r contained His^{501}, Asp^{559}, and Ser^{656}, which were conserved among this group of serine proteases. This protein was thus designated as mouse C1r. We have expressed a truncated fragment of C1r protein without the N-terminal hydrophobic sequence in *Escherichia coli* and generated a polyclonal antibody against it. Subsequent immunohistochemical analysis confirmed that mouse C1r was significantly expressed 8 d after the skin graft in both allografted and autografted skins, compared with normal skins. These collective data suggest that a component of the complement system, C1r, might contribute to the graft *versus* host immune responses in mice. *J Invest Dermatol 116:374–379, 2001*

Genetic disparities between donor and recipient are readily detected by the host immune system, resulting in host *versus* graft and graft *versus* host responses. These are manifested *in vivo* by rejection of tissue and organ grafts (Nash *et al*, 1992). The allograft response is coordinated by an interacting network of cellular and soluble mediators that result in the ultimate destruction of the foreign graft (Haeney, 1995). Of central importance to the initiation as well as perpetuation of this response, however, is the recognition of polymorphic regions of the allogeneic major histocompatibility complex (MHC) antigens by host T cells bearing clonally specific T cell receptors (Krensky *et al*, 1990; Utku *et al*, 1998). Recent studies have revealed that T cells specific for allogeneic MHC proteins expressed on transplanted cells mediate the rejection processes of allografts (Lombardi *et al*, 1989; Benichou *et al*, 1992, 1998; Fangmann *et al*, 1992). Although the critical role of T cells in the allograft response has long been demonstrated, this does not mean that antibodies, B cells, or other cells play no part in the process (Bladwin *et al*, 1995; Brauer *et al*, 1995; Merten *et al*, 1998). Despite recent advances in our understanding of the molecular mechanisms underlying T cell recognition of alloantigens, little is known concerning the actual contributions of other cells or soluble mediators involved in the graft rejection *in vivo*.

The complement system comprises a group of proteins that promote the inflammatory response (Reid and Porter, 1989). The classical pathway consists of C1, C4, and C2. The C1 macromolecular complex ($C1q:C1r_2:C1s_2$) is the product of five genes, three of them encoding the A, B, and C chains of C1q, whereas C1r and C1s are encoded by single genes (for a review, see Schmarker *et al*, 1987). Two homologous, yet distinct, serine proteases, C1r and C1s, are assembled into C1s-C1r-C1r-C1s in a Ca^{2+}-dependent manner. C1r is responsible for intrinsic C1 activation, a two-step process involving first an autolytic C1r activation and then C1r-mediated cleavage of proenzyme C1s. C1

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Reprint requests to: Dr. Tae-Yoon Kim, Room 4003, Laboratory of Dermatology-Immunology, Catholic Research Institute of Medical Science, Kangnam St. Mary Hospital, The Catholic University of Korea, 505 Banpo-dong, Seocho-gu, Seoul, Korea 137–040. Email: tykimder@cmc.cuk.ac.kr

Abbreviation: ddRT-PCR, differential display reverse transcription polymerase chain reaction.

[1]S.J.B. and Y.Y.B. contributed equally to this paper and share first authorship.

is activated upon binding antigen-antibody immune complexes containing IgM or IgG. In case of the human, C1r is a single-chain glycoprotein with a molecular weight of approximately 83,000 Da. Upon activation, it is cleaved into an A chain (M_r 56,000) and a B chain (M_r 27,000), and these two chains are held together by a disulfide linkage. Activated C1 can cleave C4 and initiate subsequent steps of the classical complement pathway. Futhermore, there is increasing evidence that antibodies can damage grafts through the activation of complement (Colletti *et al*, 1994).

Skin is considered to be highly immunogenic. A number of professional antigen-presenting cells, such as Langerhans cells and dermal dendritic cells, are involved in skin allograft rejection as well as various immunologic functions. It is likely that a variety of genes are involved in the allograft rejection as well. In this study, we initiated differential display reverse transcription polymerase chain reaction (ddRT-PCR) approaches to obtain novel transcripts in the mouse skin grafts and identified a gene clone encoding a complement C1r component. The mouse C1r was expressed to a significant level 8 d after the skin graft in both allografted and autografted mouse skins as determined by northern blot analysis and immunohistochemical analysis. In contrast, little expression was observed in normal skins. This suggests that C1r component might contribute to graft *versus* host immune responses in mice.

MATERIALS AND METHODS

Animals C3H/HeN and BALB/C male mice aged 8–12 wk were used in this study and maintained in our pathogen-free facility. Mice were fed by free access to autoclaved water and food *ad libitum*.

Skin graft Mouse skin was grafted according to a described method (Benichou *et al*, 1998). Briefly, full-thickness back skins were harvested from euthenized donor mice, the subcutaneous fat was removed, and the skin was cut to 0.5–1 cm^2 and placed in RPMI medium until used for transplantation. Recipient mice were anesthetized; skins were placed in a slightly larger graft bed prepared over the back of the recipient and stitched using silk.

For allografts, donor mice were BALB/C and recipient mice were C3H/HeN. Donor and recipient mice of autografts were BALB/C mice. The autografted and allografted skins were obtained at day 1 and 8 after skin transplantation.

Total RNA isolation and ddRT-PCR Total RNAs were isolated from the autografted and allografted skins as well as from normal BALB/C skins using TRIzol reagent as instructed by the manufacturer (Gibco-BRL, Gaithersburg, MD). ddRT-PCR was performed as described previously (Liang and Pardee, 1992) and as instructed in the Differential Display Kit (Display Systems Biotech, Los Angeles, CA) with some modifications. Briefly, each 5 μg of total RNA from the normal and grafted skins were first treated with RNase-free DNase I (Takara, Ohtsu, Japan) in the presence of 10 units of ribonuclease inhibitor (Gibco-BRL). The first-strand cDNAs were prepared from 500 ng of total RNAs using a Differential Display Kit (Display Systems Biotech) as described by the manufacturer. The RNA from each sample was incubated with one of nine downstream primers containing 11T residues and two-nucleotide anchors (AA, AC, AG, CA, CC, CG, GA, GC, and GG) for 1 h at 37°C, followed by 5 min at 95°C to inactivate the M-MLV reverse transcriptase (Gibco-BRL). Each cDNA was subjected to PCR amplification with Ex-Taq (Takara), using the original downstream primer, one of 24 10-mer 5′ primers, and [γ-^{35}S]-dATP. The 40 rounds of PCR were done at 94°C for 30 s, 40°C for 1 min 30 s, and 72°C for 1 min in a thermocycler (Perkin-Elmer Model 2400, Roche Diagnostics Systems, Mannheim, Germany), followed by a final cycle at 72°C for 10 min. The radiolabeled products were separated on a 6% DNA sequencing gel containing 7 M urea. The gel was dried and exposed on X-ray film (Fuji, Tokyo, Japan). Bands showing induced or repressed expression patterns in the allografted groups were cut out. cDNAs from bands were eluted in 100 μl TE buffer (pH 8.0). Following ethanol precipitation, the eluted cDNAs were reamplified by PCR using the same primer sets and conditions as described above. The PCR products were run on agarose gel in order to confirm the size of the cDNAs. Each reamplified band was purified by Geneclean system (Bio 101, Vista, CA). Each cDNA was subcloned into pGEM-T Easy Vector (Promega, Madison, WI) and sequenced by the dideoxynucleotide chain-termination method (Sanger *et al*, 1977). The sequences were compared with the National Center of Biotechnology Information nonredundant sequence database using the BLASTX and BLASTN programs.

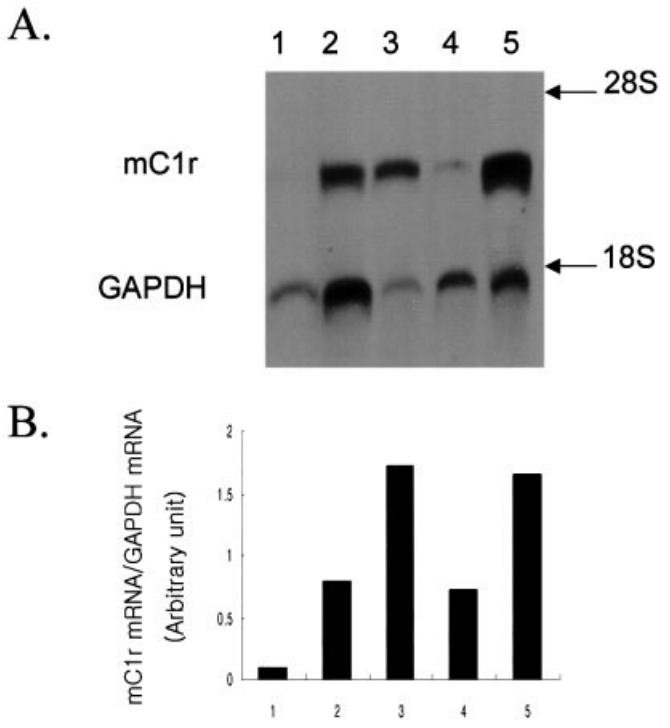

Figure 1. Northern blot analysis of the clone homologous to human complement Clr precursor. (*A*) Total RNAs were prepared from normal, autografted, and allografted mouse skin biopsies at days 1 and 8 post-transplantation. Representative hybridization signals were obtained from mC1r at the expected position corresponding to an mRNA size of 3 kb. The hybridization signal for GAPDH is shown as a control at the same blot. (*B*) Values for mC1r mRNA levels in relation to GAPDH mRNA levels in the three groups of animals. *Lane 1*, normal mouse skin; *lane 2*, autograft day 1; *lane 3*, autograft day 8; *lane 4*, allograft day 1; *lane 5*, allograft day 8.

Northern blot analysis Total RNA samples (each 20 μg) from the normal and grafted skins were electrophoresed in a 1% agarose gel containing 6% formaldehyde and 1 × MOPS buffer [1 × MOPS buffer is 20 mM MOPS, pH 7.0, 5 mM sodium acetate, and 1 mM ethylenediamine tetraacetic acid (EDTA)]. The RNA was transferred onto a nylon membrane (Schleicher & Schuell, Dassel, Germany) by capillary action and covalently fixed to the membrane by ultraviolet cross-linker (Spectrolinker, Westbury, NY). The [α-^{32}P]-labeled probes were prepared using the Prime-a-Gene Labeling System (Promega). Prehybridization, hybridization, and posthybridization wash of the membrane were carried out as described previously (Sambrook *et al*, 1989). Standardization was performed by hybridization of the same membrane with a probe from mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA. After autoradiography, quantitation of the signals was performed by densitometric analysis with Gel Doc 2000 gel documentation systems (Bio-Rad Lab, Hercules, CA).

Identification of full-length cDNA Phage cDNA library from a mouse skin in the Uni-ZAP XR vector was screened by plaque hybridization with a total of 2.5×10^7 plaques (Stratagene, La Jolla, CA). Both prehybridization and hybridization were done at 42°C. The probe was the isolated cDNA, which was labeled with [α-^{32}P]-CTP (Amersham-Pharmacia, Cleveland, OH). After two more purification procedures, the individual positive plaques were isolated using end-removed tips, and the λ-DNA purified by plaque hybridization was rescued by *in vivo* excision using an f1 helper phage. Sequences of the isolated cDNA were determined by the dideoxynucleotide chain-termination method using T7 Sequenase version 2.0 under the manufacturer's instruction (Amersham-Pharmacia).

Expression and purification of the recombinant mC1r protein The open reading frame from an mC1r cDNA clone was

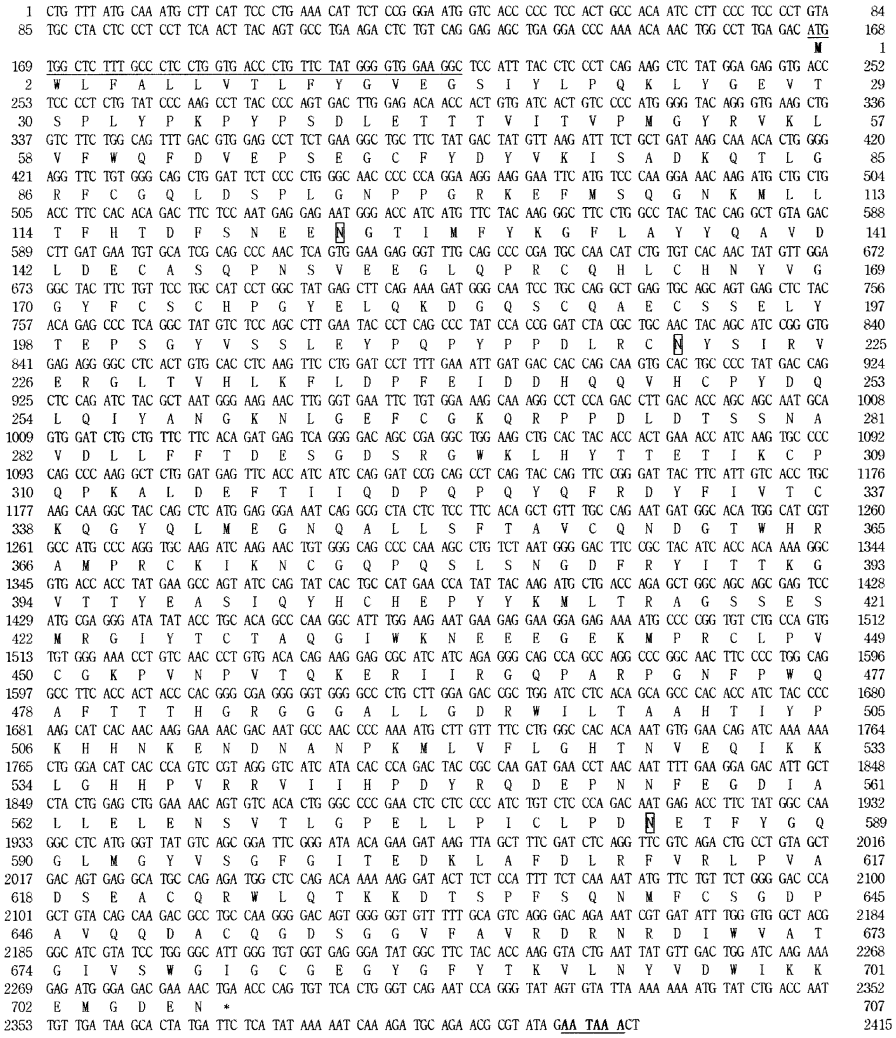

Figure 2. Nucleotide and deduced amino acid sequences of the cDNA homologous to human complement Clr precursor. The cDNA was screened from the mouse skin cDNA library. The putative signal peptide and polyadenylation signal sequences are indicated by underlining. The three boxed Asn residues are the possible glycosylation sites. The sequence is available from the GenBank, accession number AF148216

amplified by PCR with specific primers containing a restriction site (*Hind*III) added to the 5′ and 3′ ends to facilitate cloning of the PCR product. The forward primers were 5′- CTGCAGTCGACAAGCTTCATATGTGGCTCTTTGCCCTC-3′ including the signal peptide and 5′- CAGTCGACAAGCTTCATATGTCCATTTACCTCCCTCAG - 3′ without the signal peptide, and the reverse primer was 5′- ATCGATCTCGAGAAGCTTATCAACAATTGG TCAGATAC-3′. The PCR-amplified 2.2 kb cDNA was subcloned into a pET28b vector (Novagen) and introduced into *Escherichia coli* BL21 cells carrying the DE3 bacteriophage. The fidelity of the expression construct was confirmed by DNA sequencing. The protein expression and purification procedure was performed as described previously (Takai *et al*, 1998) with some modification. Upon addition of isopropyl-β-D-thiogalactoside to the culture, the mC1r recombinant protein was expressed as a fusion with histidine tagging. Induced cells were harvested by centrifugation, and lyzed by sonication within 8 M urea, 50 mM Tris-HCl, pH 8.0, and 1 mM EDTA. The supernatants were adsorbed to the nickel-chelating chromatography resin (Novagen) after clearing, and the fusion protein was eluted with 1 M imidazole, 500 mM NaCl, and 20 mM Tris-HCl, pH 7.9. The expression of the recombinant protein was confirmed by western blot analysis with T7·Tag antibody from Novagen followed by detection using the ECL plus system from Amersham (Amersham Life Science, Buckinghamshire, U.K.).

Preparation of antirecombinant mC1r antibodies Polyclonal antisera against the recombinant mC1r protein were obtained from a New Zealand White rabbit. The nickel-chelation chromatography fraction containing fused mC1r protein was resolved by 9% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The corresponding bands were excised and used to raise a polyclonal antibody in rabbits. Emulsions were prepared by mixing 0.5 ml of the Freund adjuvant (Gibco-BRL) with 0.5 ml of the excised protein solution (0.5 mg per ml) dissolved in distilled water. The emulsified antigen was injected intradermally into the back skin of a rabbit near the neck at 4 wk intervals. The rabbit was bled following the third injection and biweekly thereafter. The titer of anti-mC1r protein was evaluated by western blot analysis.

Immunohistochemistry For immunostaining of the skin biopsies from normal, autografted, and allografted mice, 4 μm cryosections were obtained from unfixed frozen tissues, allowed to air-dry for 30 min, fixed in 100% ice-cold acetone for 10 min at 20°C, and air-dried for 30 min. Sections were stored at –20°C and staining was performed as described elsewhere with minor modifications (Ashcroft *et al*, 1997). Briefly, nonspecific binding was blocked with 10% normal donkey serum. Sections were incubated for 60 min at room temperature with 1:20 diluted polyclonal primary antibody in antibody diluent reagent solution from Zymed Lab (South San Francisco, CA). Subsequently, the sections were incubated for 20 min with a biotinylated donkey antirabbit immunoglobulin (DACO, Carpinteris, CA) for 20 min, followed by horseradish peroxidase labeled avidin. 3-Amino-9-ethylcarbazole was applied as chromogen under microscopic control with an Olympus inverted microscope until the specific staining signal was visible. Control staining for each tissue was performed with the same conditions, except that preimmune rabbit serum was used as a primary antibody source.

RESULTS

ddRT-PCR and overview of the selected cDNAs Originally ddRT-PCR was performed as a method to identify novel gene(s) whose transcription is induced or repressed by the skin transplantation procedure. cDNAs from normal, syngeneic, and allogeneic grafted skins on day 1 and 8 post-transplantation were

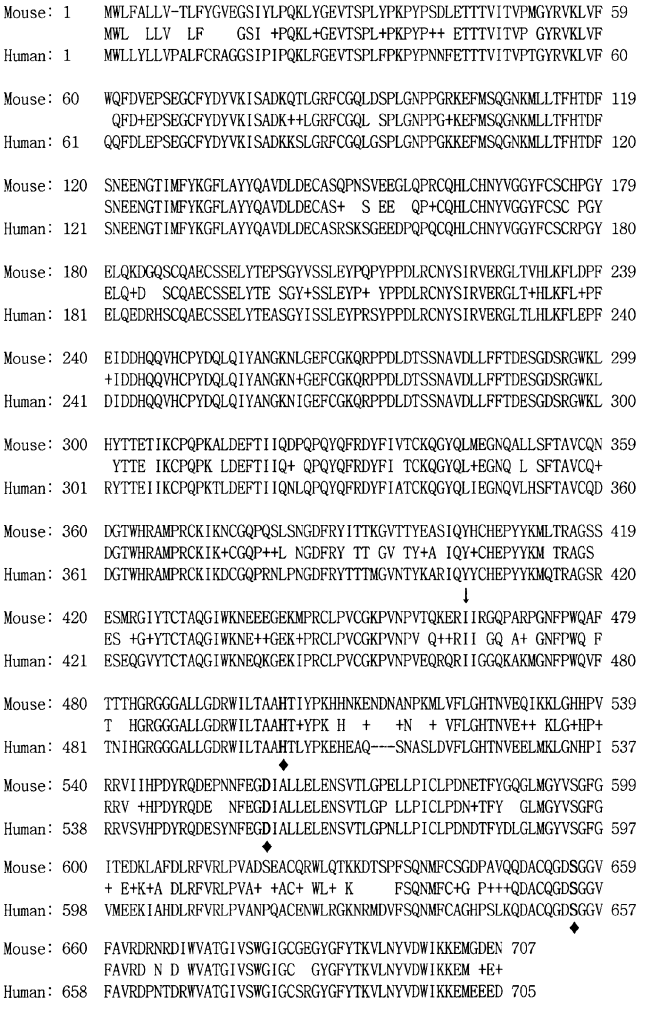

Figure 3. Sequence alignment of the amino acid sequences of the isolated mouse cDNA and human complement Clr precursor. Amino acids that are physicochemically similar are indicated by a plus symbol. Dashes in the sequences represent gaps introduced to maximize alignment. Amino acid residues marked with closed diamonds represent key amino acids conserved in trypsin-type serine protease. The solid arrow indicates the putative Arg-Ile peptide bond cleaved during the activation of mC1r.

amplified with a set of arbitrary PCR primers. The PCR products were resolved by 6% acrylamide sequencing gel containing 7 M urea and visualized by autoradiography. Eighteen candidate DNA bands were selected and isolated for further characterization. The selected cDNAs were approximately 150–400 bp in size. The reamplified PCR products were subjected to subcloning and subsequent sequencing, followed by BLAST sequence analysis (Altschul *et al*, 1990) and MasPar searches of the NCBI nonredundant nucleotide database. Out of 18 candidate genes that were sequenced and analyzed, 12 were novel sequences without a significant homology to any known genes in the database (data not shown), and the others had multiple significant hits. These six products were chosen for further characterization.

Identification of genes differentially expressed in the grafted skin Northern blot analysis was performed with total RNA isolated from the normal autografted and allografted skin using six selected partial cDNA sequences as probes (data not shown). Among the six candidate cDNAs, only one clone showing significant homology to human complement C1r precursor was highly upregulated in the grafted skin of both syngeneic and allogeneic mice (**Fig 1**). The transcript was mobilized as a band of around 3.0 kb in size similar to that of humans (Journet and Tosi, 1986). Therefore, we attempted to isolate a full-length cDNA for the mouse homolog to the human complement C1r precursor.

Screening, sequencing, and expression level of the mouse homolog to human complement C1r precursor In order to isolate a full-length cDNA encoding mouse C1r, approximately 2.5×10^7 plaques of a mouse skin cDNA library were screened using a partial cDNA fragment that was obtained from ddRT-PCR as a probe. Eight positive phage clones were selected in the initial screening. Two further rounds of phage purification were performed and a clone containing the longest DNA insert was identified from the eight positive clones with an insert 400–2400 bp in size by southern blot analysis (result not shown) and was designated mC1r. A pBluexcript phagemid of the mC1r from this clone was constructed by *in vivo* excision and was used to determine the nucleotide sequence. The phagemid obtained contained a cDNA insert approximately 2.4 kb in size that shared a high sequence homology to human complement C1r precursor (Journet *et al*, 1986; Leytus *et al*, 1986). The mC1r had an open reading frame of 2121 bp that started with a translation initiation site at position 167 and could encode a polypeptide with 707 amino acid residues with a calculated molecular weight of 80,732 Da, including a typical signal peptide sequence of 16 amino acids at the N-terminus. The nucleotide and deduced amino acid sequences of mC1r are shown in **Fig 2**. The deduced amino acid sequence of mC1r has an 89% similarity and 81% identity to that of human complement C1r precursor (**Fig 3**). This cDNA was identified as the mouse homolog of human C1r. Its RNA level is rarely detectable in a normal skin sample as shown in **Fig 1**. When the grafted skin samples at the time points of day 1 and 8 post-transplantation were subjected to northern blot analysis, however, the RNA level was strongly upregulated. mC1r mRNA levels were very similar in the autografted and allografted skin according to the densitometric analysis with GAPDH and mC1r (**Fig 1*B***). This suggests that mC1r is likely to play a pivotal role in graft *versus* host responses following the graft procedure in mice.

***In vitro* expression of a recombinant mC1r protein** From preliminary studies, our attempts to express in *E. coli* the full-length cDNA containing the hydrophobic signal peptide with a size of 707 amino acid residues resulted in only a small amount of recombinant protein appearing as insoluble aggregates (data not shown). In order to increase the level of protein expression, we prepared a truncated construct of 691 amino acid residues without the N-terminal hydrophobic putative signal peptide sequence. The recombinant protein was expressed at a relatively high level in the cytosolic fraction and could be purified by His-Tag chromatography under denaturing conditions with urea. His-Tag fused protein had migrated to a band of about 85 kDa in an SDS-PAGE analysis, which is in agreement with what we calculated from the cDNA sequence plus the extra 41 amino acid residues from the pET28b vector. Immunoblot analysis (data not shown) using T7 Tag antibody confirmed that the 85 kDa was, in fact, the His-Tag mC1r. We directly used the recombinant fusion protein in the preparation of antibody. The 85 kDa band was excised and used to raise polyclonal antibody against mC1r.

Antibody against recombinant C1r protein and immunohistochemistry In order to confirm expression of C1r in the grafted skin, a polyclonal antibody was raised against the recombinant His-Tag-fused mC1r protein. The raised polyclonal antibody was shown to be highly specific for the recombinant protein (data not shown). To confirm the expression of mC1r in the skin biopsies harvested from autografted and allografted mice, immunohistochemistry was performed using an anti-mC1r polyclonal antibody. On analyzing the skin sections harvested on day 8 following graft from both autografted and allografted mice, we found positive staining for mC1r, particularly in the dermis tissue in the allografted skin (**Fig 4*B***), as well as in the autografted

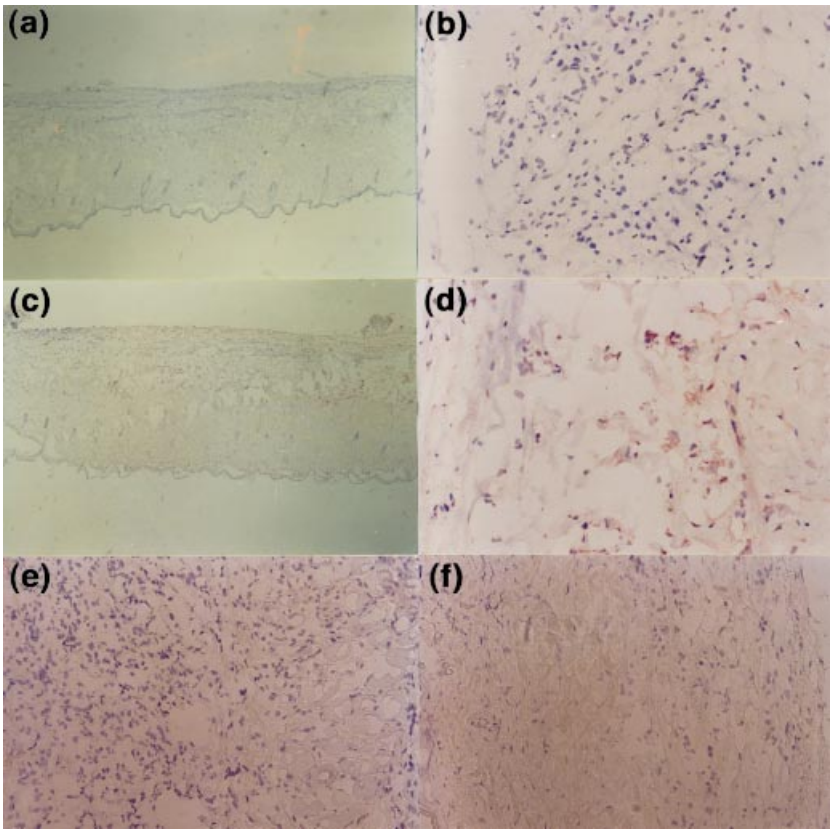

Figure 4. Expression of mC1r in normal skin tissue and allografted skin tissue on day 8 post-transplantation. The skin biopsies harvested from normal (*a*, *b*) and allografted (*c*, *d*) mice were evaluated by immunohistochemistry using an anti-mC1r polyclonal antibody, which was raised against the recombinant mC1r protein, and preimmune rabbit serum as a control antibody [*e* (normal skin tissue) and *f* (allograft skin tissue 8 d post-transplantation)]. Magnification: *a*, *c*, 40×; *b*, *d*, *e*, *f*, 200×.

skin tissue (data not shown), demonstrating that the skin is associated with a strong inflammation response. mC1r was rarely detectable in the skin from normal mice, however (**Fig 4*A***). This suggests that an increase in mC1r expression plays an important role for graft *versus* host immune responses in mice.

DISCUSSION

A series of gene products has been suggested to be involved in graft *versus* host immune responses. We have performed ddRT-PCR in order to identify genes that are differentially expressed in grafted mouse skin. From this procedure, 18 candidate cDNAs were isolated, cloned, and sequenced, and a sequence similarity search was performed. The results of a homology search revealed that six of these cDNAs had a similarity to known genes, whereas the remaining 12 cDNAs displayed no significant similarity to any deposited sequences. These six cDNAs were used as probes on northern blots to detect a differential expression, although only one showed a difference in its RNA level after the skin graft procedure. The sequence of this cDNA has a high similarity to that of human Clr. By northern blot analysis, we have identified the highly induced expression of its 3.0 kb transcript in the skins on day 8 post-transplantation. The full length of the cDNA designated as mClr was isolated from a mouse skin cDNA library using a probe of partial cDNA from ddRT-PCR. It contained no poly (A) tail region and was in accordance with the size of the transcript that was detected as a 3.0 kb range by northern blot. By comparison, human complement Clr precursor contains 705 amino acids, whereas the mClr encodes a protein with 707 amino acid residues, including a typical signal peptide sequence of 16 amino acid residues at the N-terminus. The deduced amino acid sequence of the mClr had 81% identity and 89% similarity with that of humans. The His^{501}, Asp^{559}, and Ser^{656} amino acid residues, conserved in trypsin-type serine proteases, were also conserved in the mClr as well as the primary structure of the regions surrounding these key amino acid residues. The putative N-glycosylation sites (N-X-S/T) of the mClr were identified as Asn^{124}, Asn^{220}, and Asn^{583}, and the cleavage of A and B chains is likely to occur between Arg^{462} and Ile^{463}. In addition, the number and position of the conserved cysteine residues of serine proteases in the mClr completely matched those of the human complement Clr precursor. The expression of mClr was upregulated in the grafted mouse skins. We have previously shown that type 1 cytokines, such as interleukin-2 (IL-2), interferon-γ (IFN-γ), and IL-12, play an important role in skin allograft rejection. A large variety of cells, including monocytes/macrophages, polymorphonuclear cells, fibroblasts, epithelial cells, keratinocytes, endothelial cells, and adipose cells, synthesize complement components when cultivated *in vitro* (Gasque *et al*, 1993). This biosynthesis is constitutive and is enhanced following stimulation by cytokines, such as IL-1β, IL-6, IFN-γ, and tumor necrosis factor α (TNF-α). Several reports have demonstrated that IFN-γ induces increased biosynthesis of Clr (Legeodec *et al*, 1997). Taken together, our results coincide with these findings that stimulation by IFN-γ and the expression of complement mClr are correlated (Hyun *et al*, 1998). Thus, the components of the classical complement system, C1q, C1r, C1s, C2, and C4, were upregulated after stimulation with IFN-γ. We suggest that grafted skin tissues may constitute a local source of these components and therefore they could be implicated in inflammatory or physiopathologic processes developed in grafted skin in mice.

From the immunohistochemical study, both autografted and allografted skin tissues were associated with the strong inflammatory process, and the mC1r mRNA and protein were significantly detectable in the grafted skin tissues, particularly in the dermis rather than epidermis of the skin, even on day 1 post-transplantation, suggesting that this complement component is involved in the early response to the skin graft procedure. The complement system is one of the four major proteolytic cascades of the blood. Together with the immunoglobulins, the complement system traditionally represents the humoral system of the immunologic host defense, and is involved in many biologic activities contributing to defense against microbial invaders and to inflammatory reactions (Moore,

1994). It has been proposed, however, that immune complexes initiate inflammatory responses either via activation of the complement system (Colten, 1994) or, alternatively, by the direct engagement and activation of FcR-bearing inflammatory cells (Ravetch and Clynes, 1998). Although the concept of immune-complex-triggered inflammation via activation of the complement cascade is well established, recent studies in FcR-deficient mutant mice have promoted the different view that immune complexes induce inflammation predominantly through FcR engagement, with complement proteins subserving primarily immunoregulatory functions (Ravetch and Clynes, 1998). It has been proposed that the role of the complement system is largely immunoregulatory and minimally inflammation (Matis and Rollins, 1995). Thus, the expressed mC1r may be involved in this complex graft *versus* host immune response. *In vitro* stimulation of a number of different cell types with IFN-γ by infiltrating T cells stimulates expression and production of complement components, such as C1q, C1r, C1s, C2, and C4. Therefore, the upregulation of the C1r protein level in completely different skin graft reactions is expected. This is the first result indicating the involvement of this potent classical pathway for various physiologic responses in graft *versus* host immune responses in mice.

In summary, we cloned mouse Clr homologous to the human complement Clr precursor, which plays an important role in the initiation of the activation of the classical pathway. Additionally, the expression of complement component was upregulated in the allografted and autografted skin tissues. Further study is required to elucidate, in detail, the fucntion of mClr in graft *versus* host immune responses.

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