Biochemical and Biophysical Research Communications **282,** 200–206 (2001)
doi:10.1006/bbrc.2001.4550, available online at http://www.idealibrary.com on IDEAL®

Genes Highly Expressed in the Early Phase of Murine Graft-versus-Host Reaction

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Received February 14, 2001

Graft-versus-host reaction (GVHR) is a complex process initiated upon allorecognition. For detection of early molecular events in GVHR, we first assessed time courses with respect to symptoms and serum interferon (IFN)-γ levels and then used the differential display method to compare gene transcript patterns during the early phase between acute lethal GVHR mice and syngeneic controls. In the GVHR mice, high expression levels of seven genes encoding the following molecules were detected: TGTP/Mg21 (an IFN-γ-related signaling molecule), vitronectin, Nedd5 (a mammalian septin), manganese superoxide dismutase, activin βC subunit, PRCC (a papillary renal cell carcinoma-associated molecule), and an uncharacterized gene corresponding to a mouse expressed sequence tag (EST). The expression levels of most genes peaked before the symptomatological onset and the peak of IFN-γ levels. Thus, gene expression monitoring may characterize the inductive process of GVHR and aid in the development of gene-based diagnostics and therapies. © 2001 Academic Press

Key Words: **graft-versus-host reaction; early phase; gene expression; differential display method; TGTP/Mg21; vitronectin; Nedd5; manganese superoxide dismutase; activin βC subunit; PRCC.**

Graft-versus-host reaction (GVHR), the major complication occurring after hematopoietic stem cell transplantation, transfusion, or solid organ transplantation such as intestinal or hepatic grafts, is a complex process initiated upon allorecognition of host histocompatibility antigens by donor lymphocytes (1). To date, GVHR has been characterized symptomatologically, histologically, and immunologically. In humans, diagnosis and treatment for GVHR have been carried out based on clinical signs and histologic findings (2). Cellularly defined methods have also been developed as assays for prediction of GVHR (3, 4). However, the reliability and specificity of such approaches are limited (5–7). To improve the clinical outcome further, novel strategies for investigation of GVHR are needed. Studies on molecular events occurring during GVHR have the potential to provide further insight into the pathogenesis of this disease and the discovery of targets of diagnostic and therapeutic application. There have been various reports on molecular analyses of GVHR (8–14). Although most of these approaches have been confined to selected molecules of interest, GVHR is most likely mediated by a variety of known as well as unknown molecules.

For most genes, expression is regulated at the level of transcription. Comparative analysis of gene transcript patterns under various conditions is attractive as a strategy for the discovery of a spectrum of molecular factors involved in pathological mechanisms. The differential display method (DD) is a technique that provides a rapid visualization of specific banding patterns of gene fragments by RT-PCR with anchored 3′ primers and arbitrary 5′ primers to allow comparison between two cell populations or between various organs (15). DD has been used successfully to identify inducible or repressed genes in a variety of diseases involving immunological and/or inflammatory mechanisms (16–20). However, there has been no report on the global analysis of gene expression patterns in GVHR.

To identify genes relevant to the pathogenesis of GVHR, we performed DD for *in vivo* specimens in a murine transplant model. For detection of genes whose expression levels change prior to clinicopathological manifestations, we first assessed the time course with respect to symptoms and serum interferon (IFN)-γ levels and then prepared the optimal short-term model for gene expression analysis of acute lethal GVHR using

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0006-291X/01 $35.00

DD. As a result, seven genes were found to be upregulated in the GVHR mice, which may be involved into the inductive process of GVHR.

MATERIALS AND METHODS

Our study was carried out in accordance with the Guide for Animal Experimentation for the Faculty of Medicine (University of Tokyo).

Induction and clinical monitoring of GVHR. C57BL/10 (termed B10; $H2^b$) and BALB/c ($H2^d$) mice were purchased from SLC (Japan). Donors and recipients were female and 8 weeks of age at the time of transplant. BALB/c recipients received 8.5 Gy total body irradiation delivered by X-ray at a dose of 0.45 Gy/minute. For induction of GVHR, 2×10^7 B10 donor spleen cells were intravenously infused into irradiated recipients. To obtain syngeneic controls, 2×10^7 BALB/c donor spleen cells were infused into irradiated recipients.

First, we prepared three GVHR mice and three syngeneic controls. Symptoms and survival were monitored daily after donor cell transfer. The body weight of each mouse was also measured daily. All surviving mice were observed until day 21 after the donor cell transfer. Secondly, we prepared nine GVHR mice and nine syngeneic controls. At each time point for RNA sampling, three GVHR mice and three syngeneic controls were sacrificed.

Evaluation of serum IFN-γ levels. In the preliminary experiments for clinical monitoring of GVHR, serum samples were taken from the tail veins of mice daily. In the experiments for gene expression analysis, serum samples were collected by cutting the axillary sites at the time of sacrifice of the mice. Serum IFN-γ levels were measured using a mouse interferon-γ ELISA system (Biotrak, Amersham).

DDRT-PCR. Total RNA was extracted from liver tissues using a commercial kit (Trizol, Life Technologies) according to the manufacturer's instructions. The differential display RT-PCR (DDRT-PCR) was performed using a commercial kit (Delta Differential Display kit, Clontech) as recommended by the manufacturer, except for several modifications for the nonradioactive assay. Total RNA 2 μg was reverse transcribed in 100 μl of reaction mixture using MMLV reverse transcriptase and the oligo (dT) primer. Control reactions were performed in the absence of reverse transcriptase. The cDNA was amplified by PCR using the combination of 5′ arbitrary primers (P1-P10 primers) and 3′ anchored primers (T1-T9 primers) (Clontech). Each DDRT-PCR product was electrophoresed on a 5% denaturing polyacrylamide (acrylamide:bis-acrylamide = 19:1) /8 M urea gel in 1 × Tris-borate-EDTA buffer at 2000 V for 2 h.

Collection of cDNA bands and reamplification. The gel was silver stained after electrophoresis according to the manufacturer's protocol (Promega) (21). Gel slices containing differential cDNA bands were cut out. The cDNA was eluted by incubation of the gel slices in 40 μl of 10 mM Tris–HCl buffer (pH 8.5) at 95°C for at least for 10 min. The eluted cDNA was reamplified by PCR with the same primers used in the original DDRT-PCR in 40 μl reaction mixture containing 100 μM dNTPs, 1 × PCR buffer, and 1 unit of Taq polymerase. The thermal cycling steps for PCR included denaturation at 94°C for 10 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 68°C for 2 min, and a final extension at 68°C for 7 min.

Purification of cDNA fragments with the SSCP-like procedure. The cDNA bands obtained by DD frequently contain a mixture of PCR products of similar sizes. The cDNA fragments in the reamplified mixture derived from the same DD band can be separated due to difference in the single-strand conformation of nucleic acids (22). We carried out the single-strand conformation polymorphism (SSCP)-like procedure to purify cDNA fragments (23). The reamplified PCR products were mixed with a denaturing solution containing 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol FF. The mixtures were denatured at 95°C for 5 min, immediately cooled on ice, and electrophoresed on a 6% polyacrylamide gel in 0.5 × Tris-borate-EDTA buffer at 200 V for 1–1.5 h. After electrophoresis, the gel was silver stained. Among separate bands derived from the same PCR products, major bands were cut out. The cDNA in each gel slice was eluted, amplified by PCR, and checked by electrophoresis on a 1–2% agarose gel or a 6% polyacrylamide.

Sequencing and homology search. The cDNA fragments purified with the SSCP-like procedure were subjected to either direct cycle sequencing (ABI 377 or ABI PRISM 310, PE Biosystems) or TA cloning performed using a commercial kit (Invitrogen) according to the manufacturer's instructions. The nucleotide sequences obtained were compared with known sequences by searching the EBML/GenBank database with the NCBI BLAST program (National Center for Biotechnology Information, Bethesda, MD).

Semiquantitative RT-PCR. On the basis of the obtained sequences, specific primers to amplify cDNA fragments were designed. To allow comparison of RT-PCR products derived from different samples, the concentration of cDNA in each sample was adjusted to yield equal amounts of PCR products when amplified by β-actin primers (forward primer: 5′-ATGGATGACGATATCGCT-3′, and reverse primer: 5′-ATGAGGTAGTCTGTCAGGT-3′) as internal controls. Serial twofold dilutions of the calibrated cDNA were used as templates for PCR. The thermal cycling steps for PCR involved an initial denaturation step at 94°C for 10 min, followed by 20 to 38 cycles which consisted of denaturation at 94°C for 30 s, annealing at the optimum temperature for each set of primers for 30 s, and extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The PCR products were analyzed by electrophoresis on a 6% polyacrylamide gel followed by staining with SYBR Gold (Molecular Probes). To determine the optimum number of PCR cycles for semiquantitative analysis of each gene, the correlation between the number of cycles used and the densitometric values of the bands was examined using an image analyzer and its software package for detecting linear range (Molecular Imager FX and Quantity One, Bio-Rad). Calculation for semiquantitative analysis was based on the comparison of serial dilutions of the template cDNA adjusted in concentration, resulting in equal densitometric values of PCR products between different samples. The mean expression level of each gene in three syngeneic control mice sacrificed on day 2 after donor cell transfer was used as a control (onefold) for the relative evaluation of the gene expression levels between different individuals.

The two-way analysis of variance (two-way ANOVA) was used for the evaluating changes in body weight and serum IFN-γ levels in the preliminary experiments for clinical monitoring. The two-sample *t*-test was used for comparing serum IFN-γ levels and gene expression levels between different groups of mice in the experiments for gene expression analysis.

RESULTS

Clinical Courses after Donor Cell Transfer

The GVHR mice exhibited severe symptoms such as reduced activity, abnormal gait, ruffled fur, shivering, and diarrhea between day 4 and day 6 and then died on day 7 or 8 after donor cell transfer, while the syngeneic controls exhibited only mild and reversible reduction of activity and were alive at least until the final observation on day 21. Irreversible loss of weight, which is typical of severe GVHR, was observed in the GVHR mice. In contrast, the weight loss observed in the syngeneic controls was reversible. The weights of the GVHR mice decreased to a greater extent than did those of the syngeneic controls (data not shown). Serum IFN-γ levels of the GVHR mice were equal to those

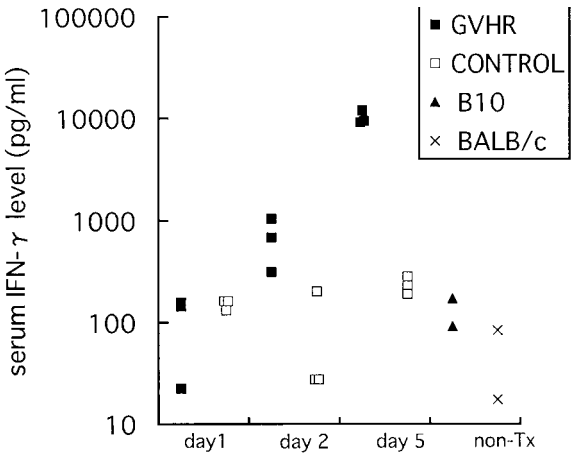

FIG. 1. Serum IFN-γ levels at each time point for RNA sampling. The serum IFN-γ levels of the GVHR mice and the syngeneic controls on day 1 after donor cell transfer were equal to those of nontreated B10 and BALB/c mice. The results of the comparison of serum IFN-γ levels between the GVHR mice and the syngeneic controls were consistent with those of the preliminary experiments for clinical monitoring (data not shown). Non-Tx indicates nontreatment.

of the syngeneic controls on day 1 after donor cell transfer, but began to increase on day 2, and peaked on day 5. Such changes in the serum IFN-γ levels were not observed in the syngeneic controls (data not shown).

GVHR Model for Gene Expression Analysis

Three time points, day 1, day 2, and day 5 after donor cell transfer, were set for sampling of total RNA of liver, which is one of the target organs in GVHR, on the basis of the assessment of time courses with respect to symptoms and serum IFN-γ levels as described above. To confirm that GVHR has occurred, serum IFN-γ levels were measured at each time point of sacrifice of the mice for RNA sampling (Fig. 1). The results were consistent with those of the preliminary experiments for clinical monitoring as described above.

Identification of Genes Highly Expressed in the Early Phase of GVHR

For investigation of early molecular events preceding clinicopathological manifestations, we applied DD to the comparison of gene transcript patterns on day 2 after donor cell transfer between the GVHR mice and the syngeneic controls. The differential display RT-PCR (DDRT-PCR) using 50 primer sets provided 33 intense cDNA bands and one weak cDNA band for the GVHR mice (Fig 2A). Of all cDNA fragments contained into these bands, 25 fragments could be reamplified by PCR. After purification using the SSCP-like procedure, the sequences of six cDNA fragments were determined by direct sequencing while those of the 19 other cDNA fragments were analyzed through TA cloning (Fig. 2B). Homology search revealed that 20 cDNA fragments corresponded to known genes, three corresponded to mouse expressed sequence tags (EST), and that two had no homology with any reported sequence.

Semiquantitative RT-PCR analysis showed that seven of the 25 cDNA fragments corresponded to genes highly expressed in the GVHR mice on day 2 after donor cell transfer, namely, genes encoding TGTP/Mg21, vitronectin, Nedd5, manganese superoxide dismutase (Mn-SOD), activin βC subunit, and PRCC, and an uncharacterized gene (termed CL-160 in this study) having a sequence completely homologous to the mouse EST AA657046 (Fig. 2C and Table 1). The expression levels of TGTP/Mg21, vitronectin, Nedd5, Mn-SOD, activinβC subunit, PRCC, and CL-160 in the GVHR mice were approximately 29-fold ($P < 0.02$), 22-fold ($P < 0.05$), 5-fold ($P < 0.02$), 4-fold ($P < 0.001$), 13-fold ($P < 0.001$), 10-fold ($P < 0.01$), and 13-fold ($P < 0.001$), as high as those in the syngeneic controls, respectively.

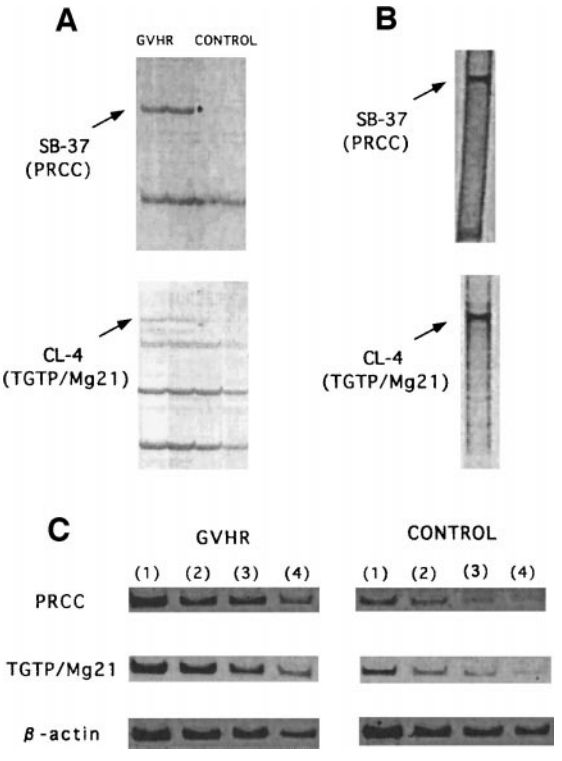

FIG. 2. Analysis using DD followed by semiquantitative RT-PCR. (A) DDRT-PCR products of gene transcripts of liver on day 2 after donor cell transfer between the GVHR mice and the syngeneic controls. For the GVHR mice, SB-37 and CL-4 were viewed as intense bands on the gel. (B) Major bands obtained using the SSCP-like procedure for purification of cDNA fragments. The cDNA eluted from the SB-37 and CL-4 bands was reamplified, and subjected to sequence analysis. Homology search revealed that SB-37 and CL-4 corresponded to PRCC and to TGTP/Mg21, respectively. (C) Verification of genes highly expressed in the GVHR mice. RT-PCR was performed using specific primers (shown in Table 2) and the optimum number of cycles (30 cycles for PRCC and 25 cycles for TGTP/Mg21) for semiquantitative analysis. (1), (2), (3), and (4) represent four serial dilutions of the cDNA concentration adjusted by amplification of β-actin, namely, 1×, 0.5×, 0.25×, and 0.125×, respectively.

TABLE 1

cDNA Fragments Corresponding to Genes Highly Expressed in GVHR Models

cDNA fragments	Length	Homology	Accession No.	Gene	Category/Feature
SB-37	730 bp	98%	AF273750	mouse PRCC	papillary renal cell carcinoma translocation-associated gene; proline-rich
CL-4	859 bp	99%	L38444, U15636	mouse TGTP/Mg21	IFN-γ-induced 47 kDa GTPase; expressed independently of IRF-1
CL-7	915 bp	98%	D49382	mouse Nedd5	mammalian septin; having GTPase activity and interaction with actin-based filaments
CL-67	481 bp	98%	U95962	mouse activin βC subunit	cytokine; TGF-β superfamily
CL-69	302 bp	99%	M77123	mouse vitronectin	extracellular matrix component
CL-151	341 bp	96%	L35525	mouse Mn-SOD	antioxidant enzyme
CL-160	363 bp	100%	AA657046	mouse EST	corresponding to an uncharacterized gene

Note. GVHR indicates graft-versus-host reaction; IFN, interferon; IRF, interferon regulatory factor; TGF, transforming growth factor; Mn-SOD, manganese superoxide dismutase; EST, expressed sequence tag.

The primers used for the semiquantitative RT-PCR analysis of these genes are listed in Table 2.

Time Courses of Gene Expression Levels

The time courses of mRNA expression levels of the seven genes were analyzed by semiquantitative RT-PCR analysis (Fig. 3). All of the genes were expressed differentially between the GVHR mice and syngeneic controls on day 5 as well as on day 2 after donor cell transfer. On day 5, the expression levels of TGTP/Mg21, vitronectin, Nedd5, Mn-SOD, activinβC subunit, PRCC, and CL-160 in the GVHR mice were approximately 11-fold ($P < 0.005$), 9-fold ($P < 0.01$), 3-fold ($P < 0.001$), 3-fold ($P < 0.002$), 6-fold ($P < 0.002$), 3-fold ($P < 0.005$), and 5-fold ($P < 0.02$), as high as those in the syngeneic controls, respectively. The expression levels of the genes were not significantly different between the GVHR mice and the syngeneic controls on day 1.

In the GVHR mice, the peak expression level of each gene, except for the gene encoding vitronectin, was observed on day 2 after donor cell transfer. The expression level of vitronectin on day 5 was near in value to that observed on day 2, and the expression level on day 1 was lower than on day 2 and day 5.

In the syngeneic controls, a relatively weak peak of Mn-SOD expression level was observed on day 2 after donor cell transfer while the highest expression levels of vitronectin, activinβC subunit, and PRCC were observed on day 1. The difference in the expression levels of TGTP/Mg21, Nedd5, and CL-160 between the three time points was not significant.

DISCUSSION

To our knowledge, this is the first report of the molecular analysis of GVHR using DD. In previous studies, RNA used for DD was extracted from either *in vivo*

TABLE 2

Primers Used for Semiquantitative RT-PCR

Gene	Sequence of primer set	Annealing temperature	PCR product size
TGTP/Mg21	5′-GCAGGCCAGAGGATTCAT-3′ 5′-ATATACTTTGCTGTCATATTTGCACGA-3′	54°C	124 bp
Vitronectin	5′-CAGGACAGCGAGATTCCAGAA-3′ 5′-TTGCACTGCTCCATGTAGTCG-3′	57°C	240 bp
Nedd5	5′-GGCAGGGATTTACGTTTAATTCAG-3′ 5′-GAGCAAGTATCTGTTAGCAAAAGC-3′	57°C	429 bp
Mn-SOD	5′-GAGCTGAGAGTTCTATGTCCT-3′ 5′-TGATGGTTTGTATATTCTTGGGCC-3′	55°C	185 bp
Activin βC subunit	5′-GAACAAAATGACATTGCTTGTGGTAGG-3′ 5′-GAGGCCTGCGGGTGTAGTTAG-3′	59°C	285 bp
PRCC	5′-CTTCTCCCGGAAACCTTCAGA-3′ 5′-TGGGGACAGGGTAAGGGTATG-3′	58°C	281 bp
CL-160	5′-GCTGGAAGACAGTTCTGCTAC-3′ 5′-CATGGAGTTCAGGAAGCCAAG-3′	57°C	276 bp

Note. Mn-SOD indicates manganese superoxide dismutase.

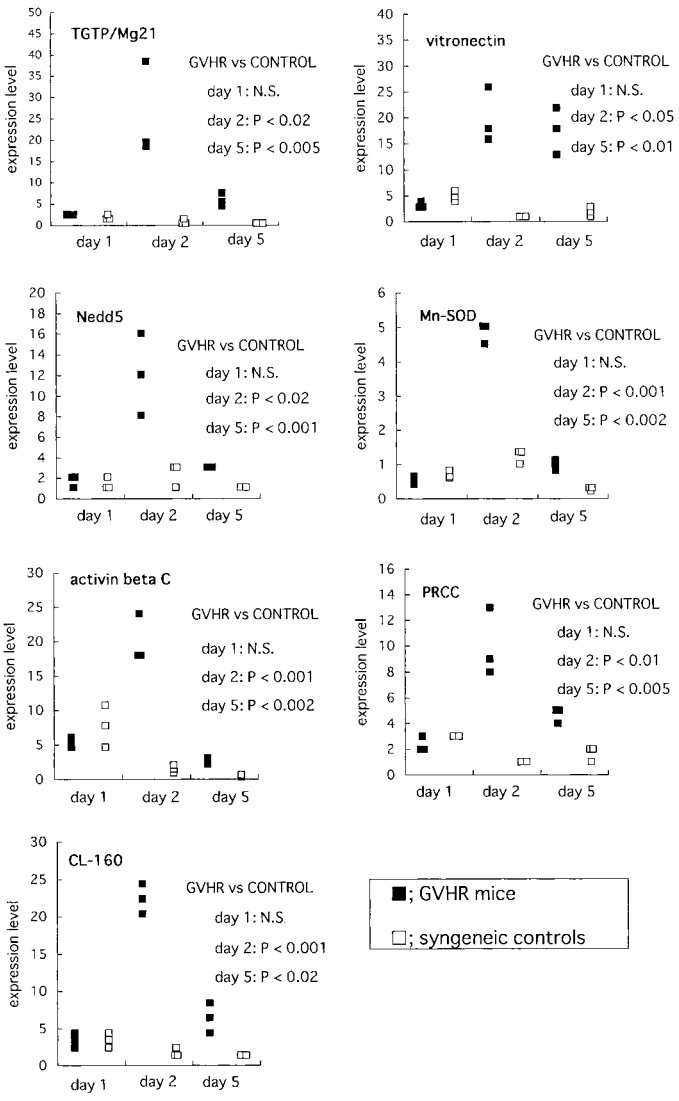

FIG. 3. Changes in gene expression levels over the time course of GVHR. On day 2 and day 5 after donor cell transfer, the expression levels of seven genes in the GVHR mice were higher than those in the syngeneic controls. On day 1, the difference in their expression levels between the GVHR mice and the syngeneic controls was not significant. In the GVHR mice, the expression level of each gene peaked on day 2 (TGTP/Mg21: $P <$ 0.05 vs day 1 or day 5, Nedd5: $P < 0.02$ vs day 1 and $P < 0.05$ vs day 5, Mn-SOD: $P < 0.001$ vs day 1 or day 5, activinβC subunit: $P <$0.005 vs day 1 and $P < 0.002$ vs day 5, PRCC: $P < 0.02$ vs day 1 and $P < 0.05$ vs day 5, and CL-160: $P < 0.001$ vs day 1 or day 5), except for that of vitronectin. The lowest expression level of vitronectin was observed on day 1 ($P < 0.01$ vs day 2 or day 5), and the expression level on day 2 was not significantly different from that on day 5. In the syngeneic controls, the expression level of Mn-SOD peaked on day 2 ($P < 0.02$ vs day 1 and $P < 0.01$ vs day 5), but the expression level was much lower than in the GVHR mice. The highest expression levels of three genes were observed on day 1 (vitronectin: $P <$ 0.01 vs day 2 and $P < 0.05$ vs day 5, activin βC subunit: $P < 0.05$ vs day 2 and $P < 0.02$ vs day 5, and PRCC: $P < 0.001$ vs day 2 and $P < 0.05$ vs day 5). The difference in the expression levels of TGTP/Mg21, Nedd5, and CL-160 between the three time points was not significant.

specimens or *in vitro* cultured cells derived from clinical or experimental samples (16–20). *In vivo* specimens most frequently consist of heterogeneous cell populations. In particular, cellular profiles differ between distinct lesions defined clinically or histologically as inflamed and noninflamed lesions. In these cases, some of the genes detected by DD may encode molecular markers specific to histologically predominant cell types in the lesion. Analysis of mRNA of cultured cells by DD can identify genes whose expression is induced or repressed under various conditions. However, it is possible that DD occasionally fails to detect genes relevant to pathogenesis due to unexpected modifications of expression patterns as a result of culture procedures. Considering these points, we compared gene transcript patterns of *in vivo* specimens of target organs prior to clinicopathological manifestations between the GVHR mice and the syngeneic controls using DD.

GVHR is regarded as an immunopathologic process involving two consecutive phases, the "afferent phase" and "efferent phase" (1). In the typical form of GVHR, clinicopathological features are observed in the efferent phase but not in the afferent phase. On the other hand, the afferent phase is characterized by altered cytokine networks in which Th1-type cytokines such as IFN-γ are predominant but without clinical signs or histologic changes. The pathological aspects in target organs during the afferent phase are less well understood. Analysis of molecular events in target organs during the afferent phase may help elucidate the processes leading to the induction of GVHR and aid the discovery of early diagnostic markers or targets for preemptive therapies prior to clinical manifestations.

The application of DD for *in vivo* specimens in the early time point after donor cell transfer could identify seven genes upregulated during the afferent phase of GVHR. These genes have not been described in previous reports on GVHR, and some of them are known to be involved into the mechanisms of immune response, inflammation, cell turnover, and tissue remodeling as described below.

TGTP/Mg21, a member of the IFN-γ-induced 47-kDa GTPase family, has been cloned as an upregulated gene after stimulation of macrophages with IFN-γ or during T cell development (24, 25). In contrast to the IFN-γ-induced 65-kDa GTPase family members, TGTP/Mg21 and other members of the 47-kDa GTPase family are induced by IFN-γ independently of IFN-regulatory factor 1 (IRF-1) (26). Although IFN-γ is thought to play an important role during the development of GVHR, previous reports on its functional aspects in GVHR are obviously conflicting (13, 27–29). To clarify the role of IFN-γin the pathogenesis of GVHR, signaling molecules dominating differentially the complex cellular response to IFN-γ, such as the 47-kDa and 65-kDa GTPase families, should be investigated further.

Vitronectin is a multifunctional glycoprotein existing in blood and in tissues as an extracellular matrix component (ECM) (30). Other ECMs, such as fibronectin and tenascin, have been reported to be related to

GVHR (31, 32). Altered expression of ECMs including vitronectin may contribute to tissue damage of GVHR through their effects on immune response, inflammation, and tissue remodeling (33–35).

Nedd5 gene belongs to the family of mammalian septins and is ubiquitously expressed in various tissues (36). Septins are evolutionarily conserved cytoskeletal GTPases that can form heteropolymer complexes involved in cytokinesis and other cellular processes (36). Cytoskeletal organization-related genes are upregulated during T cell activation (37). The increased expression of Nedd5 in the liver of the GVHR mice may also reflect cytoskeletal alteration of target organs during the afferent phase of GVHR.

Mn-SOD is induced by the superoxide anion, endotoxin, irradiation, and inflammatory cytokines (38, 39). Induction or overexpression of Mn-SOD can protect cells against damage caused by tumor necrosis factor (TNF)-α or oxidants (40, 41). However, overproduction of hydrogen peroxide by Mn-SOD could lead to cell death or induce effector molecules degrading tissues (38, 42). The increased expression of Mn-SOD in the GVHR mice may not only reflect its induction associated with altered cytokine networks or with oxidative stress, but is also a subject for debate on the functional relevance of the oxidative stress-related cascade to GVHR (43, 44).

Activin βC subunit is a member of the activin family that belongs to the transforming growth factor (TGF)-β superfamily (45). Whereas activin βA and βB subunit genes are expressed in various tissues, activin βC and βE subunit genes are expressed mainly in liver (45). A recent study suggests that the biological functions of activin βC and βE subunit are different from those of activin βA subunit (46). Therefore, the pathophysiological significance of the increased expression of activin βC subunit in the liver of the GVHR mice might differ from that of increased expression of activin βA subunit in several inflammatory diseases (47, 48).

PRCC gene has been identified as a gene involved into the chromosomal translocation in human papillary renal cell carcinoma (49, 50). Although it is speculated that PRCC may function in a signaling cascade because it possesses a proline-rich domain, its physiological and pathophysiological roles still remain uncertain (50). The increased expression levels of PRCC in the liver of the GVHR mice, as well as high expression of Bcl-2 and PML in the target tissues of GVHR, seem to make the functional relevance of some cancer-related genes to GVHR worth investigating (51, 52).

A portion of the sequence of the cDNA fragment termed CL-160 was 100% identical to the mouse EST AA657046 corresponding to a putative but as yet uncharacterized gene. Efforts to characterize the gene fragment are ongoing.

Changes in the expression levels of some genes were observed in the syngeneic controls. This may reflect the influence of irradiation or irradiation-associated inflammatory mediators on the expression of these genes (39, 53). However, since such changes in the syngeneic controls were negligible, the changes in the expression levels of the seven genes over the time course of GVHR are considered to be dominated by alloreactivity. Notably, in the GVHR mice the expression levels of most of the genes peaked before the symptomatological onset and the peak of serum IFN-γ levels. Thus, gene expression monitoring may characterize the inductive process of GVHR without the need for symptomatology or histology, aiding in the development of gene-based diagnostics and therapies. Recent studies show the possibility that gene expression profiling can be used to categorize or characterize diseases (54, 55). The expression patterns of functionally related genes have been observed to be similar (56). Aspects of the expression of certain gene families or cascades suggested by the present study may present candidates to be molecular targets of clinical application and a framework for functional studies on the molecular pathogenesis of GVHR.

ACKNOWLEDGMENT

A part of this work was supported by NEDO (New Energy and Industrial Technology Development Organization, Japan).

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