Differential expression of human Fas mRNA species upon peripheral blood mononuclear cell activation

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Human Fas/Apo-1 is a cell-surface protein that mediates apoptosis upon ligation with Fas ligand. The gene lies on the long arm of chromosome 10, consists of nine exons, and spans more than 26 kb of DNA. We previously reported the presence of a Fas variant mRNA, designated as FasΔTM, in human peripheral blood mononuclear cells. FasΔTM is generated by alternative splicing of the intact exon 6, which encodes the Fas transmembrane domain. In the present study, we describe three novel forms of Fas mRNA that are generated by alternative splicing of exons 3, 4, 6 and 7. These three mRNA variants undergo a frameshift and produce truncated polypeptides because of the appearance of a stop codon in the altered open reading frame. On activation of the peripheral blood mononuclear cells, a decreased expression of alternatively spliced Fas mRNA species correlated with increased cell-surface expression of Fas. These results suggest that differential expression of alternatively spliced Fas mRNAs may play a role in regulation of Fas function via regulation of the production of the membrane-bound and the soluble, secreted Fas protein products.

INTRODUCTION

Both mouse and human *fas* cDNA have been cloned [1–3]. DNA sequence analysis reveals that the *fas* cDNA that encodes the Fas molecule consists of a leader peptide at its N-terminus, an extracellular domain comprising three cysteine-rich subdomains, a hydrophobic transmembrane domain, and a cytoplasmic tail at its C-terminus [1–4]. Activation of Fas, either by antibody-induced cross-linking or by interaction with its ligand, is required for induction of apoptosis [1–7]. Structural similarity places Fas in the nerve growth factor (NGF) receptor/tumour necrosis factor (TNF) receptor superfamily [1–3]. Recently, we cloned the human *fas* gene and characterized its exon–intron organization and promoter region [8]. The single gene encoding human Fas lies on the long arm of chromosome 10, consists of nine exons, and spans more than 26 kb of DNA [8–10].

The *fas* gene is expressed in several tissues in human and mouse, including thymus, spleen, ovary and heart, and on a number of cell types, including activated T- and B-lymphocytes [1–3,11,12]. The *lpr* gene, which is associated with lymphoproliferation and autoimmune disease, was initially described as a *fas* mutation in CBA-*lpr*^{cg} mice [13]. We and two other independent groups have previously described abnormal Fas mRNA transcripts caused by insertion of a retroviral early transposable element in intron 2 of the *fas* gene in MRL-*lpr*/*lpr* mice [14–16]. Aberrant splicing of the Fas mRNA transcript with insertion of a 25 nucleotide fragment of unknown identity has also been described in rat liver [17]. Recently, we described a variant form of Fas mRNA species in human peripheral blood mononuclear cells (PBMCs) [18]. This Fas mRNA variant is produced by splicing out of an exon which encodes the Fas transmembrane domain. We demonstrated that this variant encodes the soluble form of the Fas molecule. This soluble Fas molecule can block anti-Fas-induced apoptosis *in vitro* and alter lymphocyte development and proliferation in response to self antigen *in vivo*. Elevated levels of this soluble form of Fas were found in the serum of some patients with the autoimmune disease, systemic lupus erythematosus.

In this report, we describe the characterization of the spectrum of alternatively spliced Fas transcripts in human PBMCs and the identification of three other previously undefined spliced sequences. We have developed a reverse transcription (RT)-PCR assay for quantification of Fas mRNAs in the cells and used this assay to examine the expression of Fas mRNAs in the PBMCs upon activation. The results provide evidence that alternative splicing plays an important role in the regulation of *fas* gene expression.

MATERIALS AND METHODS

Isolation and culture of PBMCs

PBMCs were separated by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.) density-gradient centrifugation from freshly drawn heparinized venous blood from a normal individual. The cells were resuspended to 2×10^6/ml in RPMI 1640 supplemented with 10% (v/v) fetal-calf serum, 2 mM glutamine, 100 units/ml penicillin and 10 μg/ml streptomycin. Approximately 4×10^5 (200 μl) of the cells were then cultured with or without supplementation with phytohaemagglutinin-P (PHA-P) (10 μg/ml; Pharmacia, Piscataway, NJ, U.S.A.) in a flat-bottomed 96-well plate (Costar Corp., Cambridge, MA, U.S.A.) in 5% CO_2 at 37 °C.

PCR product cloning and nucleotide sequencing

The PCR products were analysed by electrophoresis on a 1.2% agarose gel (Seakem GTG agarose; MFC Corp.) and recovered from a 0.8% low-melting-temperature agarose gel (NuSieve

Abbreviations used: PBMC, peripheral blood mononuclear cell; PHA-P, phytohaemagglutinin-P; NGF, nerve growth factor; TNF, tumour necrosis factor; RT, reverse transcription.

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The nucleotide sequence data reported are available from EMBL/GenBank under accession nos. X83490 to X83493.

GTG agarose; MFC Corp.). The products were directly subcloned into a pCR vector (Invitrogen, San Diego, CA, U.S.A.) following the procedures recommended by the supplier. *Escherichia coli* that were transformed with the recombinants containing these DNA fragments were plated; 40 transformants were randomly selected from approximately 10^4 colonies to prepare plasmid DNA. The inserts of these 40 plasmids were excised by *Eco*RI and electrophoresed in a 1.0% agarose gel. The templates for nucleotide sequencing were alkali-denatured plasmids which were purified using QIAGEN 100 tips (QIAGEN Inc., Studio, CA, U.S.A.) following the procedure recommended by the supplier. All nucleotide sequences were derived from both strands of each insert and determined by the dideoxy chain termination method using modified bacteriophage T7 DNA polymerase [19] (United States Biochemical Corp., Cleveland, OH, U.S.A.). Oligonucleotides derived from the *fas* cDNA sequence were used to obtain the overlapped sequence. Nucleotide sequencing data were analysed using the Sequence Analysis Software Package of the University of Wisconsin Genetics Computer Group (Madison, WI, U.S.A.).

RNase protection assay

The *fas* cDNA corresponding to nucleotides from 565 to 767 was amplified by using 20 ng of plasmid DNA of the cloned full-length *fas* cDNA in pCR™ vector and primers, 5′-*GGAATTC*ATACCAAGTGCAGATGGT A-3′ (nucleotides from 565 to 582) and 5′-*ATCAAGCTT*CACCCAAACAA-TTAGT GG-3′ (complementary to nucleotides from 747 to 767). Underlined nucleotides represent *Eco*RI and *Hin*dIII restriction sites in the sense and antisense primers, respectively. Italics are used to indicate nucleotides not derived from the *fas* cDNA sequence. Amplification was carried out for 30 cycles. Each cycle consisted of 60 s denaturation at 94 °C, 90 s annealing at 50 °C, and 60 s extension at 72 °C. The amplified *fas* cDNA was purified after agarose gel electrophoresis, digested with *Eco*RI and *Hin*dIII, and subcloned into pGEM-7Zf (+). Transcription reactions were performed using 1 μg of *Eco*RI-linearized DNA template, 50 μCi of [α-^{32}P]CTP (Amersham Corp., Arlington Heights, IL, U.S.A.) and 20 units of SP6 RNA polymerase (Transcription *In Vitro* Systems; Promega), according to the instructions of the supplier. The reaction was terminated by the addition of 2 units of RQ1 RNase-free DNase (Promega). The radiolabelled antisense RNA probe was 258 nucleotides in size, of which 203 nucleotides were complementary to Fas mRNA at the nucleotides from 565 to 767 and the other 55 nucleotides were complementary to the vector sequence between the SP6 transcription initiation start site and the *Hin*dIII cloning site.

RNase protection experiments were performed using the RPA II kit (Ambion, Austin, TX, U.S.A.), according to the instructions of the supplier. Briefly, approximately 8×10^4 c.p.m. of antisense RNA probe was ethanol-precipitated with 25 μg of cellular RNA from PBMCs after PHA-P stimulation for 24 h. The resulting pellets were dried and resuspended in 20 μl of hybridization buffer containing 80% formamide. The mixture was heated at 90 °C for 3 min and then incubated for 16 h at 42 °C. RNase digestion was performed by adding 200 μl of a 1/100 dilution of a stock solution containing 250 units/ml of RNase A and 10000 units/ml of RNase T1 and incubating at 37 °C for 30 min. RNase-resistant fragments were ethanol-precipitated, resuspended in 8 μl of loading dye containing 80% formamide, electrophoresed on a 6% polyacrylamide/7 M urea gel, and visualized by autoradiography for 36 h at −70 °C. Negative controls included in each experiment consisted of mixtures of antisense RNA probe and 10 μg of yeast tRNA.

Semi-quantitative RT-PCR and Southern blot analysis

Total cellular RNA was isolated from 2×10^5 cells using the guanidine isothiocyanate/acid phenol method [20] and subjected to first-strand cDNA syntheses in a total volume of 33 μl using the first-strand cDNA synthesis kit (Pharmacia P-L Biochemicals Inc., Milwaukee, WI, U.S.A.) by the recommended procedure. Two oligonucleotides, 5′-CACTTCGGAGGATTGCTCAACA-3′ (nucleotides from 170 to 191) and 5′-TATGTTGGCTCTT-CAGCGCTA-3′ (complementary to nucleotides from 1316 to 1336), were used to amplify human Fas mRNA from nucleotides 170 to 1336 [2]. The amplification was performed in a 50 μl reaction volume using 5 μl of RNA:cDNA template and 2.5 units of *Taq* DNA polymerase (Amplitaq; Perkin-Elmer Cetus, Norwalk, CT, U.S.A.) in the presence of 1.5 mM $MgCl_2$. A total of 30 cycles was carried out using a Perkin-Elmer GeneAmp PCR System 9600. Each cycle consisted of denaturation at 94 °C for 60 s, annealing at 56 °C for 90 s, and extension at 72 °C for 120 s. To ensure that nearly equivalent amounts of template were initially added in each PCR reaction, concurrent PCRs for amplification of human β-actin expression were utilized as a control. To ensure that the PCR conditions were not biased for production of one form of the cDNA, concurrent PCRs were performed with plasmids containing *fas* cDNA, or variant alone, or both mixed in various ratios. The RT-PCR-derived DNA fragments were electrophoresed on a 0.8% agarose gel and then transferred to nitrocellulose filters (Microseparation, Inc., Westboro, MA, U.S.A.) [21]. The blot was prehybridized for 4 h and hybridized for 16 h at 42 °C in 50 mM Tris/HCl buffer, pH 7.4, containing 50% formamide, 5 × Denhardt's, 1 M NaCl, 10% dextran sulphate, and 100 μg/ml sonicated salmon sperm DNA. The probe used in this analysis was a 463 bp *Xba*I DNA fragment (nucleotides from 1199 to 1661) derived from human *fas* exon 9. The probe was labelled using the random hexanucleotide priming method [22] and [α-^{32}P]dCTP (Amersham Corp., Arlington Heights, IL, U.S.A.).

Flow cytometry analysis of Fas expression

Single-cell suspensions of the PBMCs (2×10^5/sample) were stained in PBS containing 10% fetal-calf serum, 0.1% sodium azide, and 20 μg/ml mouse anti-(human Fas) monoclonal antibody (IgM) (Upstate Biotechnology, Inc., Lake Placid, NY, U.S.A.). The cells were then washed with PBS and stained with fluorescein isothiocyanate (FITC)-conjugated goat anti-(mouse IgM) (Southern Biotechnology Associates, Birmingham, AL, U.S.A.) for 30 min. As a control, the same cells were similarly treated, except that the primary antibody was replaced with an irrelevant mouse IgM. Viable cells (10000 per sample) were analysed by flow cytometry on a FACScan (Becton–Dickinson, Mt. View, CA, U.S.A.) with logarithmic scales.

RESULTS

Our previous report characterized one Fas mRNA splice variant, designated as FasΔTM, which lacked a 63-nucleotide segment encoding the Fas transmembrane domain [18]. Characterization of the corresponding gene indicated that this 63-nucleotide segment is encoded by an intact exon, namely exon 6 [8]. As shown in Figure 1, three additional relatively less prominent products were also amplified from RNA from unstimulated PBMCs from a normal individual by RT-PCR. To determine the structure and significance of these PCR products, which ranged in size from 985 bp to 857 bp, DNA fragments were recovered

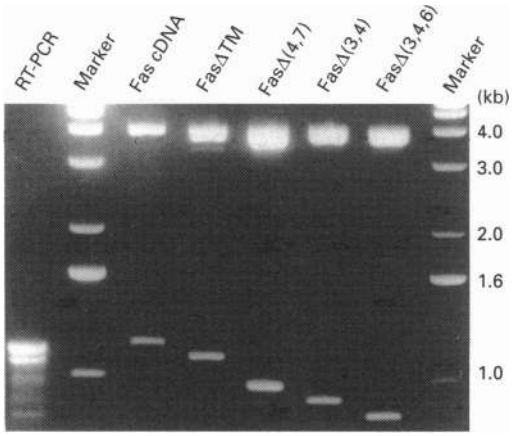

Figure 1 Agarose gel electrophoresis of *Eco*RI digests of recombinants

DNA fragments from a normal subject amplified by RT-PCR are shown. The DNA fragments were eluted from corresponding gel segments and subcloned into a pCR vector. The cloned inserts are Fas cDNA, 1167 bp; FasΔTM, 1104 bp; FasΔ(4,7), 985 bp; FasΔ(3,4), 920 bp; FasΔ(3,4,6), 857 bp. Standard DNA size markers are shown.

from the corresponding gel segments and subcloned into the pCR vector (Figure 1). The nucleotide sequence derived from each insert and the deduced amino acid sequence were compared with those of the previously reported human Fas and FasΔTM and are shown in Figure 2(a) [2,18]. A schematic representation of the patterns of the four sequences in the alternatively spliced Fas mRNAs is shown in Figure 2(b). These inserts displayed distinct internal nucleotide deletions. However, unlike FasΔTM, the alternative splicing of these exons in Fas mRNAs resulted in alteration of the Fas authentic open reading frame.

Specifically, FasΔ(4,7) had a 109-nucleotide deletion from 529-GC TTA GAA to TGC ACC AA-637, which corresponds to exon 4. This deletion gives rise to an out-of-frame amino acid sequence (Figure 2). A second 83-nucleotide deletion was present from nucleotide sequences 763-TG AAG AGA to TTA AAT CCT-845, which corresponds to exon 7. The novel polypeptide that this transcript encodes consists of 149 amino acid residues with a calculated molecular mass of 16646 Da.

FasΔ(3,4) had a 247-nucleotide deletion starting at nucleotide sequence 391-GT GAA AGG and ending at nucleotide sequence TGC ACC AA-637, which corresponds to exons 3 and 4 (Figure 2). This deletion also caused a frame shift mutation which would give rise to an altered amino acid sequence. It is capable of encoding a polypeptide of 103 amino acid residues with a calculated molecular mass of 11434 Da.

FasΔ(3,4,6) had a 310-nucleotide deletion derived from the regions described in FasΔTM and FasΔ(3,4). It is possible that this variant is generated from a Fas mRNA transcript which has undergone alternative splicing identical to that observed in FasΔTM and FasΔ(3,4) (Figure 2). If the FasΔ(3,4,6) is capable of encoding a protein product, it would represent a peptide containing only 86 amino acid residues and would have a calculated molecular mass of 9389 Da.

Hydropathy plot analysis [23] revealed that the putative polypeptides encoded by FasΔ(4,7), FasΔ(3,4) and FasΔ(3,4,6) retain the hydrophobic leader peptide of the previously reported normal Fas molecule, but lack the hydrophobic transmembrane domain in their altered amino acid sequences. The absence of the hydrophobic segment for anchoring the molecule to the membrane suggests that they might also be expressed as soluble forms and may be secreted. However, they would not retain the conformation of the Fas extracellular domain structure as observed for FasΔTM which can bind to the Fas ligand. Also, they lack the peptide recognized by the currently available monoclonal antibodies specific for human Fas. This would explained our inability to identify the protein products in COS-7 cells transfected with the recombinant expression vectors containing these cDNA inserts by either flow cytometry analysis or immunoprecipitation using these antibodies.

Northern blot analysis indicated two prominent bands at approximately 2.7 kb and 1.9 kb (results not shown) [2]. The two mRNA species are probably due to differences in the length of the 3′-untranslated region as a result of alternative use of two polyadenylation signals. They do not correspond to the variant Fas mRNAs as none of the variants has a nucleotide deletion that is sufficient to account for the 0.8 kb difference. The Fas mRNA variants cannot be detected by Northern blot analysis. The small difference of 63 nucleotides between FasΔTM and the full-length Fas cannot be adequately separated on RNA denaturing agarose gel, whereas the FasΔ(4,7), FasΔ(3,4) and FasΔ(3,4,6) splice variants may be expressed at levels that are too low to be detected by Northern blot analysis.

To confirm that Fas mRNA variants detected in PBMCs by RT-PCR were not due to a RT-PCR artifact, solution hybridization and RNase protection experiments were performed. A uniformly radiolabelled antisense RNA probe was produced from a construct containing *fas* cDNA corresponding to exons 4–7 using SP6 RNA polymerase in the presence of an [α-^{32}P]CTP (Figure 3). The probe was hybridized to total cellular RNA isolated from cells stimulated by PHA-P for 24 h, digested with RNase A and T1, and analysed by electrophoresis on a 6% polyacrylamide/urea denaturing gel. Autoradiography revealed two protected fragments that were 203 nucleotides and 135 nucleotides in length (Figure 3). As indicated in the interpretative diagram at the bottom of the Figure, the 203-nucleotide fragment was protected by the full-length Fas transcript, whereas the 135-nucleotide fragment was protected by FasΔTM, which lacks exon 6. Additional fragments of 125 nucleotides, 130 nucleotides, and 62 nucleotides, which would have been predicted to be protected by FasΔ(4,7), FasΔ(3,4) and FasΔ(3,4,6) respectively, were not observed. This observation demonstrated that the 135-nucleotide fragment protected by FasΔTM can be detected in normal human PBMCs and is not due to a PCR artifact. The lack of detectable fragments from the other three Fas splice variants indicated that they might be expressed at very low levels and are not detectable by the RNase protection assay.

In view of the low expression of the three lower-molecular-mass Fas splice variants, we developed a semi-quantitative RT-PCR assay followed by Southern blot analysis to determine the relative abundance of the Fas mRNAs prepared from PBMCs stimulated for different times with or without PHA-P. To assure that the PCR conditions were not biased for production of one form of the cDNA, concurrent PCRs were performed with plasmids containing *fas* cDNA, or the variant alone, or the two mixed in various ratios (Figure 4). The amount of products amplified by PCR correlated with the ratio of the various templates of Fas splice variant cDNA added at the initiation of the PCR reaction. These results indicated that this PCR assay can be used to detect extremely low amounts of Fas splice variants relative to the full-length *fas* cDNA.

In view of the fact that *fas* gene expression was significant increased in activated T- and B-cells [11,12], the full-length Fas and the Fas splice variant transcripts were analysed at different times after stimulation to determine if the expression of the Fas splice variant transcripts also is regulated by activation. To

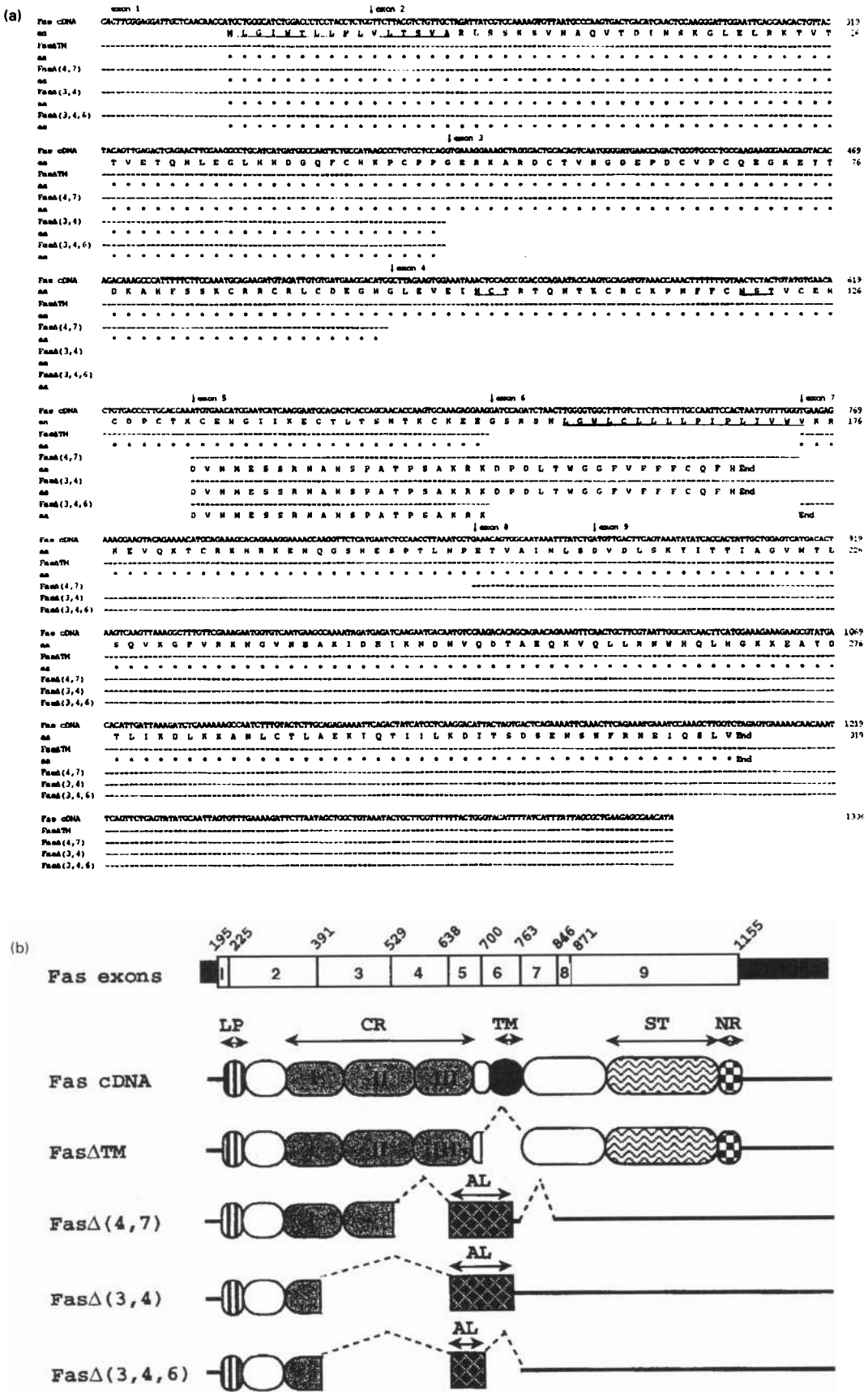

Figure 2 For legend see facing page

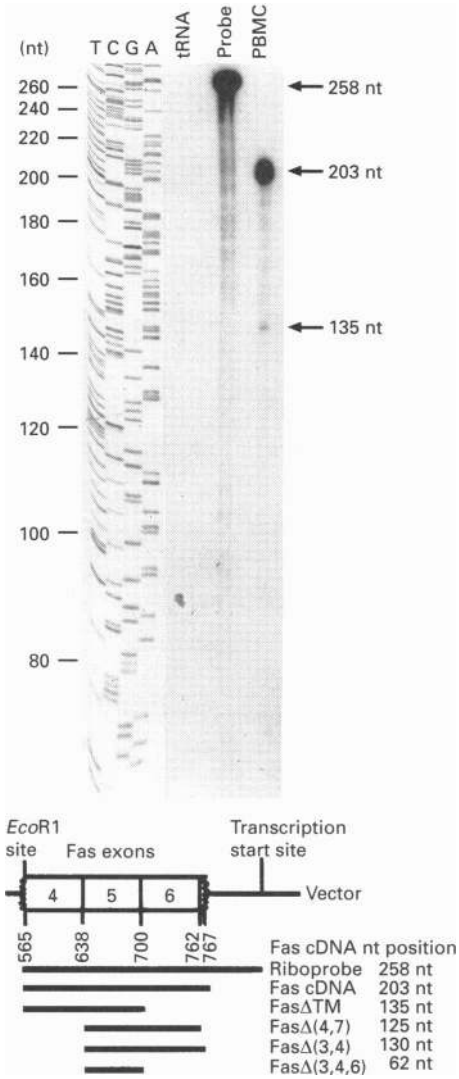

Figure 3 RNase protection analysis of Fas mRNA

Total cellular RNA was isolated from the PBMCs cultured and stimulated by PHA-P for 24 h. 25 μg of the RNA was hybridized with the [α-^{32}P]CTP-labelled antisense RNA probe corresponding to the region of a part of exon 4, the entire exons 5 and 6, and a part of exon 7. Unhybridized RNAs were digested with RNase A and RNase T1, and the resulting protected fragments were fractionated on a 6% polyacrylamide/7 M urea gel and visualized after autoradiography for 24 h. As controls, the probe was hybridized to 10 μg of the yeast tRNA and subsequently treated (lane for tRNA) or not treated (lane for probe) with RNase. The sizes of the riboprobe and PBMC mRNA protected fragments as indicated in the right margin were estimated from the M13mp18 DNA sequence ladder size marker as indicated in the left-hand margin and corrected for the 5% slower mobility of RNA fragments in the polyacrylamide/urea gel. A schematic diagram representing the Fas exon region studied, the riboprobe, and the riboprobe predicted to be protected by the Fas mRNA species is shown beneath the autoradiography. The transcription start site for SP6 transcript and the *Eco*RI restriction site used for linearization of the recombinant are indicated.

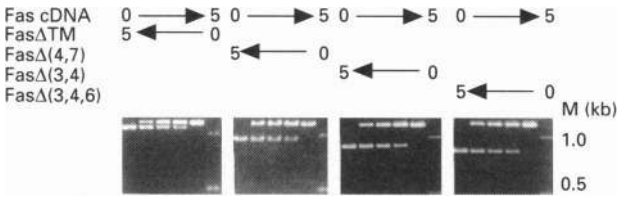

Figure 4 PCR amplification of Fas splice variant transcripts

Plasmids containing full-length Fas cDNA or Fas splice variants were mixed at the indicated concentrations. The results demonstrate the validity of the RT-PCR assay in quantitation of the relative ratios of Fas mRNAs. The Figure depicts the products of PCRs separated on a 0.8% agarose gel and stained with ethidium bromide. The PCRs were performed using a ratio of the cloned Fas cDNA to the cloned variant that varied from 0:5 to 5:0. The resulting products reflect the relative proportions of the Fas cDNA and the template added to the reaction mix. M, 1 kb ladder as the size marker.

ensure that the initial amounts of template in each PCR were added equally, concurrent PCRs for amplification of human β-actin expression were utilized as a control. The results indicated that, in resting PBMCs, there was nearly equivalent expression of the full-length Fas transcript and the FasΔTM transcript (Figure 5a). At different times after stimulation, there was a decrease of alternatively spliced forms of Fas, particularly FasΔTM, and an increase in the full-length Fas cDNA. To quantify accurately the abundance of the Fas splice variant transcripts as reflected by the copy number and not the total amount of DNA as indicated by ethidium bromide staining, the PCR products were blotted to a membrane and hybridized with [α-^{32}P]dCTP-labelled *fas* exon 9-specific probe since the exon 9 sequence is present in all of the *fas* cDNA splice variants. This analysis revealed a 4-fold increase in the production of the full-length Fas mRNA along with decreased production of FasΔTM in the cells cultured in the presence of PHA-P (Figures 5b and 5c), but not in the cells cultured in the absence of PHA-P (results not shown). Surface expression of the Fas molecule also steadily increased during PBMC activation (Figure 6), consistent with the RT-PCR result (Figure 5) and previous reports [9,12]. The RT-PCR followed by Southern blot analysis also demonstrated that the additional Fas RNA splice variants are expressed at very low levels (less than 5% in unstimulated cells and less than 1% in stimulated cells), consistent with failure of detection of these by Northern blot analysis or by RNase protection assay.

DISCUSSION

Alternative splicing in the regulation of gene expression is thought to be an important event which can control mRNA expression quantitatively or qualitatively and is an adjunct to the regulation of promoter activity (reviewed in [24,25]). In the case of the human *fas* gene, the alternative splicing generates FasΔTM and three other splice variants. These may play an important role in

Figure 2 Human Fas mRNA variants

(**a**) Comparison of the nucleotide and the deduced amino acid sequences of the four Fas mRNA variants with the sequence of full-length human Fas. Both nucleotide and deduced amino acid sequences of the four mRNA variants are aligned to the sequences of the full-length Fas [2]. The numbers on the right-hand side refer to nucleotide position and amino acid residue position as designated in [2]. Nucleotide identities are represented by dashes while amino acid residue identities are represented by stars. Deletions are represented by blanks. The altered amino acid residues are indicated. The putative leader peptide, the two potential N-linked glycosylation sites, and the putative transmembrane domain are underlined. Primer positions for amplification of the Fas mRNA are italicized. Arrows mark the first nucleotide position of exons 2–9. (**b**) A schematic representation of alternatively spliced Fas mRNA variants and their encoding proteins. The nine exons encoding the Fas molecule are shown in the upper panel. Numbers at the top refer to nucleotide positions for translation starting and ending, and exon starting. The translated and untranslated regions are indicated by boxes and thick solid lines, respectively. Regions lacking in the Fas mRNA variants are indicated by broken lines. LP, CR, TM, ST and NR represent leader peptide, cysteine-rich subdomains, transmembrane domain, signal transduction domain and negative regulation domain, respectively [4]. AL indicates the altered amino acid sequences in the Fas mRNA variants. The Roman numerals indicate three cysteine-rich subdomain repeats.

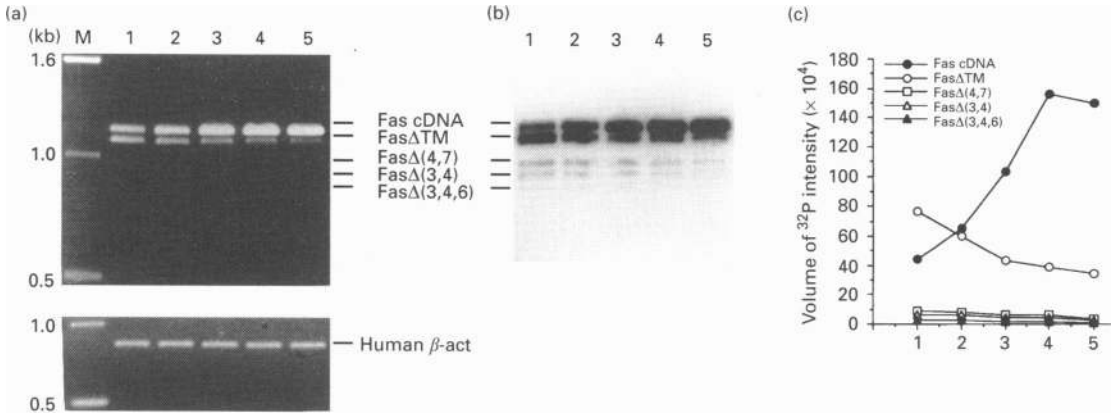

Figure 5 Fas expression in PBMCs upon activation

Total cellular RNA was isolated from the PHA-P stimulated PBMC at 0 h (lane 1), 6 h (lane 2), 12 h (lane 3), 24 h (lane 4) and 48 h (lane 5). M, 1 kb ladder as the size marker. (**a**) RT-PCR analysis of Fas mRNA expression. The total cellular RNA was subjected to semi-quantitative RT-PCR as described in the Materials and methods section. The amplified products were separated by a 0.8% agarose gel and stained with ethidium bromide. The presence of equal amounts of RNA/cDNA template subjected to PCR was verified by semi-quantitative analysis of human β-actin mRNA. (**b**) Southern blot analysis. Identity of the bands representing Fas mRNAs was verified by hybridization of the blot with the α-^{32}P-labelled Fas exon 9-specific probe. (**c**) Relative abundance of Fas mRNAs. Intensity of the bands shown in (**b**) was quantified by using a Molecular Dynamics Densitometer.

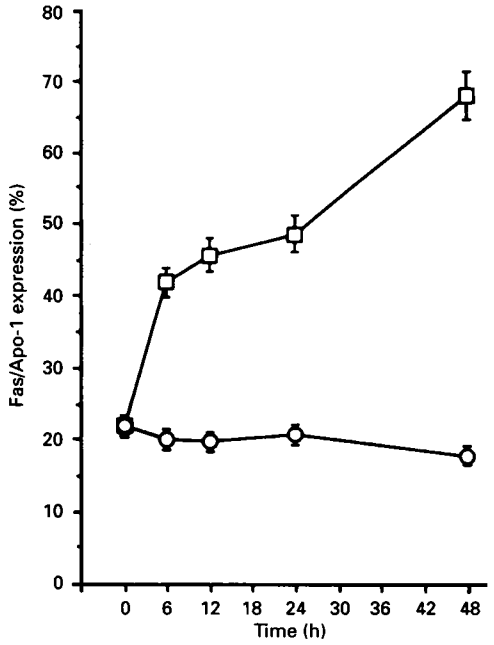

Figure 6 Flow cytometry analysis of Fas protein expression

PBMCs were collected after culture with or without PHA-P at the indicated time. Single-cell suspensions were stained with an IgM mouse monoclonal antibody specific for human Fas followed by FITC-conjugated goat anti-(mouse IgM). The percentage (mean ± S.E.M. of three independent experiments) of cells expressing a surface Fas molecule is shown. PBMCs cultured with PHA-P, open squares; without PHA-P, open circles.

regulating apoptosis mediated by the Fas–Fas ligand pathway by regulating expression of the membrane-bound and soluble forms of the Fas molecule. Our initial description of FasΔTM suggested that FasΔTM encoded a soluble form of the Fas molecule that could bind to the Fas ligand and consequently inhibit its interaction with membrane-bound Fas [18]. In this paper, we demonstrate that resting human PBMCs express nearly equivalent levels of full-length Fas and FasΔTM cDNA. After activation, there is an increase in the full-length Fas cDNA concomitantly with a decrease in the expression of the FasΔTM transcript. T- and B-cell activation have been shown to be correlated with an increased surface expression of Fas and also have been correlated with increased Fas-mediated apoptosis mediated by an autocrine or paracrine mechanism [26–28]. We propose that both down-modulation of FasΔTM and increased expression of full-length Fas enhance the ability of Fas ligand to interact with membrane-bound Fas and that this is one factor that regulates activation-induced apoptosis.

The failure to detect the other three Fas splice variants by Northern blot analysis and RNase protection assay indicated that these transcripts are present at very low abundance in the human PBMC. This concept is supported by the estimation of abundance of these transcripts determined by semi-quantitative RT-PCR followed by Southern blot analysis. Furthermore, these transcripts would all encode a truncated Fas protein which cannot bind to the Fas ligand. Thus, the significance of the presence of the other three Fas mRNA variants in human PBMCs is currently unknown. It is speculated that they may play a role in regulation of the expression of the other two transcripts by acting as a simple on/off switch, in much the same way as transcription is used to control gene expressions of the sex-lethal [29], transformer [30], and suppresser of white apricot [31] genes of *Drosophila*. After cell activation, decreased expression of these variant transcripts concomitantly with increased expression of the full-length Fas transcript supports this concept. It is interesting to note that heterozygous mutations at several points along the Fas mRNA have been associated with a lymphoproliferative autoimmune disease syndrome with increased $CD4^-CD8^-$ T-cell in children [32,33]. The mutated Fas mRNA transcript results in production of a truncated Fas molecule with altered Fas extracellular and intracellular domains. It is speculated that the truncated Fas molecule on one allele might be included into a non-functional Fas trimer on the cell surface which might bind Fas ligand but not deliver a Fas apoptosis signal. Thus, it appears to have a potent inhibitory effect on apoptosis mediated by the cell-surface Fas molecule.

Note added in proof (received 19 July 1995)

Additional forms of alternatively spliced Fas have been recently reported [34]

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