The EMBO Journal vol.9 no.12 pp.3839-3847, 1990

Activated T cells transcribe an alternatively spliced mRNA encoding a soluble form of Qa-2 antigen

Nusrettin Ulker, Keith D.Lewis, Leroy E.Hood and Iwona Stroynowski[1]

California Institute of Technology, Division of Biology, 147-75 Pasadena, CA 91125, USA

[1]Corresponding author

Communicated by M.Steinmetz

Among the best characterized non-classical mouse major histocompatibility antigens are the Qa-2 molecules. These proteins can serve as targets for allogenic cytotoxic T cells and as signal transducing molceules. They are structurally similar to H-2 transplantation antigens in their N-terminal and β_2-microglobulin binding domains but differ at their C-termini. While the H-2 antigens span the cell membrane, the Qa-2 molecules are attached to the cell surface via phospholipid anchors. The genetic information encoding this attachment is contained in exon 5. In concanavalin A activated splenocytes the expression of membrane bound Qa-2 antigens declines and, simultaneously, soluble forms of Qa-2 molecules are secreted. We demonstrate here that the soluble Qa-2 polypeptides are translated from alternatively spliced mRNAs lacking exon 5, while the membrane forms are encoded by the full-size transcripts. In cultured cells the alternative splicing of the *Qa-2* message is induced by T-cell activation with concanavalin A. The canonical mRNA encoding the membrane form of Qa-2 predominates in unstimulated mouse tissues but the cultured cell lines, like activated T cells, express enhanced levels of the truncated mRNA. In some cell lines almost all Qa-2 transcripts lack exon 5. For example, in L cells, mRNAs encoding soluble Qa-2 molecules are at least 10 times more abundant than *Qa-2* transcripts encoding phospholipid anchored antigens. These findings are discussed in terms of potential functions of membrane bound and secreted Qa-2 molecules.

Key words: activated T cells/alternative splicing/major histocompatibility complex/Qa-2 molecules

Introduction

A variety of the molecules involved in vertebrate immune recognition are encoded by the major histocompatibility complex (MHC). In mouse, these include two to three highly polymorphic class I transplantation antigens (H-2) that are expressed on most adult cells of the body. These so-called classical H-2 transplantation antigens are ~45 kd membrane bound glycoproteins which associate non-covalently with nonpolymorphic ~12 kd β_2 microglobulin (β_2m) light chains, and serve to bind antigenic peptides for presentation to T cells (Bjorkman *et al.*, 1987). The H-2 peptide presenting molecules participate in the induction of cytotoxic T-cell tolerance to self-proteins, in elimination of virally infected cells and in graft rejection (Zinkernagel and Doherty, 1980). The genes that encode the class I polypeptides are divided into six to eight exons that correlate with the structural and functional domains of these molecules. Exon 1 specifies the leader peptide; exons 2 and 3 encode N-terminal domains that bind viral and endogenous peptides; and exon 4 determines the region involved in β_2m association. The hydrophobic membrane spanning domain is encoded by exon 5 and the cytoplasmic tail by exons 6-8. Murine MHC also contains ~30 other genes that are highly homologous to the class I transplantation antigens at the DNA level and have similar exon/intron organizations. They map predominantly to the *Qa* and *Tla* subregions of MHC and encode membrane bound as well as soluble products (reviewed in Stroynowski, 1990). In contrast to the H-2, the Qa and Tla polypeptides display a very low degree of amino acid polymorphism, have restricted tissue distribution and different patterns of developmental expression. Since the structure of the Qa and Tla proteins appears compatible with peptide presentation, it has been proposed that they represent immunologically relevant molecules which bind a unique, though as yet undefined set of peptide antigens, and may potentially engage in the interactions with T-cell receptors (Stroynowski, 1990).

One of the best characterized families of non-classical MHC molecules is that which includes Qa-2 molecules. In the BALB/c mouse they are encoded by at least one gene, *Q7*, and in C57BL/10 mouse by at least two almost identical genes called *Q7* and *Q9* (Devlin *et al.*, 1985; Soloski *et al.*, 1988). The Q7 and Q9 proteins are structurally similar to the H-2 molecules in their N-terminal and β_2m binding domains but they differ at their C-termini. In H-2 antigens the C-termini span the membranes while in Qa-2 molecules they are attached to cell surfaces by a phosphatidylinositol (PI) moiety (Stroynowski *et al.*, 1987; Waneck *et al.*, 1988). The Qa-2 antigens are initially synthesized as precursor proteins containing the exon 5 encoded transmembrane domain and a short three residue, cytoplasmic tail. This C-terminal portion contains all the features necessary for the PI attachment and is believed to be cleaved off prior to the addition of the PI tail (Ulker *et al.*, 1990).

Expression of the Qa-2 antigens is highest on lymphoid cells and can be modulated by different factors. In concanavalin A (Con A) activated splenocytes, the biosynthesis of Qa-2 molecules is enhanced several-fold but the levels of the membrane bound Qa-2 antigens remain the same or are decreased (Rabinowitz *et al.*, 1986) and, simultaneously, soluble Qa-2 molecules are secreted (Soloski *et al.*, 1986). We have shown previously that both membrane bound and soluble molecules are synthesized from the same gene in transfected cell lines and hypothesized that the soluble form is generated from the membrane antigen by post-translational cleavage with endogenous PI-phospholipases or other proteases (Stroynowski *et al.*, 1987). In this manuscript we demonstrate that soluble and cell surface forms of Qa-2 molecules are translated from two different, alternatively

spliced mRNAs. The preference for the alternative splicing of exon 5 and the concomitant 'switching' from PI-membrane bound to secreted form of Qa-2 is dependent on activation of the T cells. We also report here that low level expression of both types of *Qa-2* transcripts is detectable in many non-lymphoid tissues and cells. These findings are discussed in the light of hypothetical functions of the Qa-2 molecules.

Results

Soluble Qa-2 molecules are synthesized by L-cell Q9 transfectants expressing mutant integral membrane Qa-2 antigens

Transfection of the C57BL/10 *Qa-2* genes, *Q7* or *Q9*, into the hepatoma lines (Hepa-1) leads to the expression of two forms of the Qa-2 proteins: the major product is a PI-linked ~40 kd membrane bound antigen (mQa-2), and the minor product is a ~39 kd soluble Qa-2 molecule (sQa-2) (Soloski *et al.*, 1988). These two forms are indistinguishable by biochemical criteria from the cell surface and secreted Qa-2 proteins produced by resting and Con A activated splenocytes. Surprisingly, the *Qa-2* transfected L cells synthesize only the ~39 kd sQa-2 form (Stroynowski *et al.*, 1987 and Figure 1). The absence of mQa-2 form was proposed to be due to the inability of L cells to process membrane destined PI-linked proteins. Moreover, it has been hypothesized that the L cell sQa-2 form, and by inference, the molecules secreted from T cells and transfected hepatomas, represent immature intermediates derived from the processing of Q7 and Q9 precursors. In the course of analyzing structural features responsible for the PI modification of Q9 antigen, we have generated a series of mutants encoding integral membrane molecules (Ulker *et al.*, 1990). These mutants carry substitutions of amino acids encoded by exon 5 or have additional amino acids attached to their carboxyl ends. Biochemical analyses of L cells expressing the integral membrane Q9 antigens demonstrated that the transfectants still synthesized the ~39 kd sQa-2 forms. The data for one such L cell transfectant is shown in Figure 1. *Q9#3* construct encodes a cell surface molecule containing 15 extra C-terminal residues and the corresponding mutant Q9#3 protein can be precipitated from ^{125}I-labeled L cells as a ~42 kd protein (Figure 1b). In addition, sQa-2 molecules are detectable in the supernatants of Q9#3 transfected [^{35}S]methionine-labeled L cells (Figure 1a). The secreted Qa-2 molecules are produced in similar quantity and are identical in size to the soluble forms found in the supernatants of L cells transfected with the wild-type *Q9* gene (*wtQ9*). To exclude the possibility that the sQa-2 forms from L cells are caused by 'leakiness' of the *Q9#3* mutant, we have analyzed expression of *Q9#3* gene in transfected Hepa-1 cells. Immunoprecipitation of ^{125}I cell surface labeled Hepa-1 transfectants revealed the presence of one species of mQa-2 molecule, ~42 kd in molecular weight (Ulker *et al.*, 1990). All of the mQa-2 molecules on Hepa-1 *Q9#3* transfectants were resistant to PI-phospholipase C (PI-PLC) suggesting that they represent exclusively integral membrane forms of the Q9 antigen. In contrast, *wtQ9* transfected Hepa-1 cells expressed only the ~40 kd, PI-PLC sensitive molecules. Both transfectants secreted small quantities of sQa-2 proteins with the relative molecular weight of ~39 kd (data not shown). Thus, the soluble form of Qa-2 in *Q9#3* cell transfectants cannot be attributed to the leakiness of this mutant.

We considered the possibility that sQa-2 is generated by shedding or by protein processing of the mQa-2 form and that the size difference is due to a cleavage of the mQa-2 C-terminus or to differences in glycosylation. To test these hypotheses ^{125}I-labeled L cell transfectants were chased in cold medium for 8 h. The cells and the culture supernatants were then harvested and equal cell equivalents were analyzed by immunoprecipitation with Qa-2 specific monoclonal antibody (mAb) 20-8-4. No decrease in the level of mQa-2 was observed and no sQa-2 was detected (data not shown). These results argue against precursor–product relationship between sQa-2 and mQa-2 in the transfected cells.

To test whether differential N-linked glycosylation is responsible for the molecular weight difference between the two forms of Qa-2 proteins, ^{125}I-labeled and PI-PLC treated spleen cells and their supernatants were immunoprecipitated along with ^{35}S-labeled culture medium of either *wtQ9* (data not shown) or *Q9#3* transfected L cells (Figure 2). Immunoprecipitates were divided into two equal fractions of which one was treated with enzyme Endo F while the other was left untreated. Analysis of the samples on SDS–PAGE revealed that both Qa-2 forms carry two

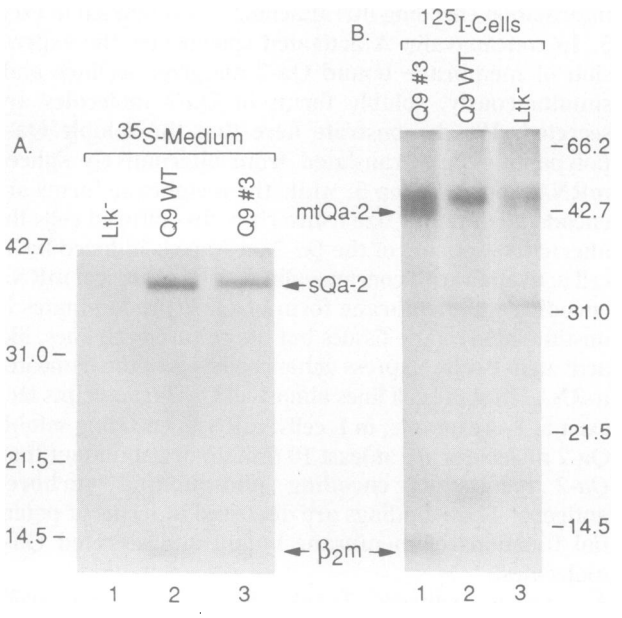

Fig. 1. Ltk$^-$ cells transfected with a *Q9#3* mutant construct synthesize an integral membrane form of Qa-2 as well as a secreted molecule. **(A)** Ltk$^-$ cells stably transfected with *Q9* wild-type (*Q9wt*) or *Q9#3* mutant were metabolically labeled with [^{35}S]methionine for 10 h. The cell-free culture medium from untransfected Ltk$^-$ cells (lane 1), *Q9wt* (lane 2) and *Q9#3* transfectants (lane 3) were then immunoprecipitated with Qa-2 specific monoclonal antibody (mAb) 20-8-4. Immunocomplexes were analyzed on a 12% SDS–PAGE. The positions of ~39 kd and ~12 kd proteins corresponding to soluble Qa-2 (sQa-2) and β_2 microglobulin (β_2m) are indicated by arrows. **(B)** The cell lines in (A) were radiolabeled by lactoperoxidase catalyzed iodination. Cell lysates were prepared and NP40 soluble material from untransfected Ltk$^-$ cells (lane 3), *Q9wt* (lane 2) and *Q9#3* transfectants (lane 1) were analyzed by immunoprecipitation with mAb 20-8-4 as described in (A). The positions of mol. wt markers are indicated. Membrane bound mutant Qa-2 (mtQa-2) is marked by an arrow. The origin of ~45 kd band which immunoprecipitates unspecifically from all ^{125}I-labeled cells is not known.

N-linked carbohydrate groups as predicted from *Q9* gene sequence (Devlin *et al.*, 1986). The difference of 1–2 kd in the molecular weights of the unglycosylated core polypeptides and the fact that class I molecules lack O-glycosylation are consistent with the hypothesis that the difference between sQa-2 and mQa-2 is likely to reside in the primary protein structure.

Selective splicing of exon 5 accompanies Con A induced T-cell activation and cell specific expression of soluble and membrane Qa-2 forms

To test whether the two types of Qa-2 molecules could arise from two alternatively spliced transcripts, we analyzed exon usage in the 3′ end of the *Q9* gene in the transfected cells and in the resting/activated splenocytes by the polymerase chain reaction (PCR). The *Q7/Q9* gene specific primers for PCR (Figure 3a) were chosen from the sequences bordering exons 5–8 because of the probability that the differences will reside in the C-terminus of the protein. This hypothesis is based on the observation that both Qa-2 forms are reactive with N-terminus specific mAb 20-8-4, both bind β_2 microglobulin and differ from each other in their ability to be processed by exon 5 dependent PI enzymes. Based on the previously reported Qa-2 cDNA sequences derived from spleen (Stienmetz *et al.*, 1981; Devlin *et al.*, 1986) and liver (Lalanne *et al.*, 1985) we predicted that PCR amplification of the cDNA fragment encoding mQa-2 antigen will produce a single ~480 bp product, designated here as *mQa-2*. Indeed, this fragment is the predominant PCR product made from C57BL/10 resting splenocytes RNA (Figure 3b). In contrast, L cells transfected with *wtQ9* or *Q9#3* constructs synthesize smaller transcripts that give rise almost exclusively to ~360 bp DNA fragments, designated here as *sQa-2* (Figure 3b, line 5). Con A activated CB7BL/10 splenocytes that express high levels of mQa-2 and sQa-2 molecules give rise to ~480 bp as well as ~360 bp PCR products. The sQa-2 product constitutes ~40% of the total amplified DNA (Figure 3b, lane 4). Hepa-1 cells transfected with *Q9* gene transcribe both alternatively spliced forms of mRNA (data not shown). Untransfected L cells and spleen cells from BALB/c Kh-H-2^{dm2} mice which lack the genes encoding

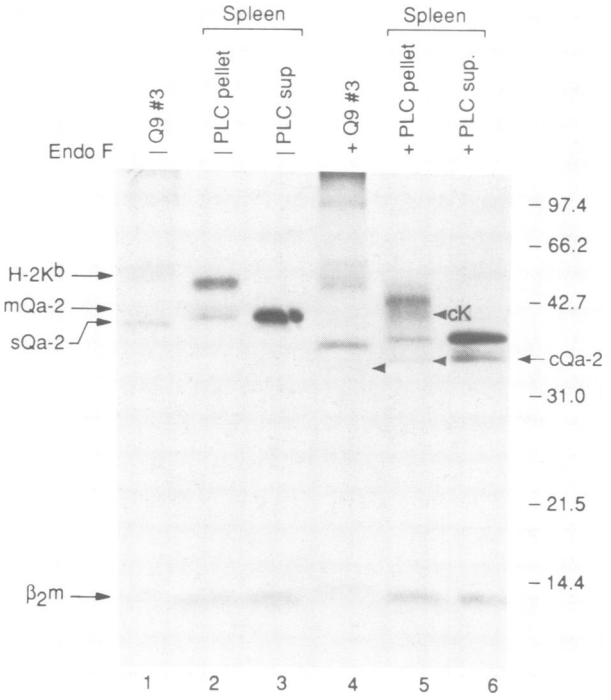

Fig. 2. N-linked glycosylation is not responsible for the molecular weight difference between the PI-linked and the secreted Qa-2 molecules. [^{125}I]-labeled resting spleen cells from C57BL/10 mice were treated with 0.1 U PI-PLC for 1 h at 37°C. Cells and medium were then harvested by centrifugation. [^{35}S]methionine labeled cell-free culture medium of *Q9#3* transfectants shown in Figure 1 were utilized as a source of the secreted Qa-2 molecule. Immunoprecipitation with mAb 20-8-4 was carried out on the culture medium of the *Q9#3* transfectants (lanes 1 and 4), and PI-PLC-treated pellet (lane 2) or a supernatant (lane 3) of C57BL/10 cultured spleen cells. Immunocomplexes were then divided into two equal fractions. While one fraction was treated with 0.02 U Endo F for 1 h at 37°C, the other fraction was left untreated. Both fractions were then analyzed by SDS–PAGE as indicated in the legend to Figure 1. mAb 20-8-4 coimmunoprecipitates transplantation antigen H-2K^b (indicated by an arrow) with the Qa-2 molecules. The double bands of the Qa-2 molecules seen in lanes 4, 5 and 6 are due to the incomplete removal of the glycan moieties by Endo F treatment. cK and cQa-2 indicate the core polypeptides for H-2K^b and Qa-2, respectively. The relative mobility difference seen in the β_2m is due to the allelic differences between C57BL/10 and C3H mice from which the Ltk$^-$ cell line was derived. Mol. wt markers are shown on the right.

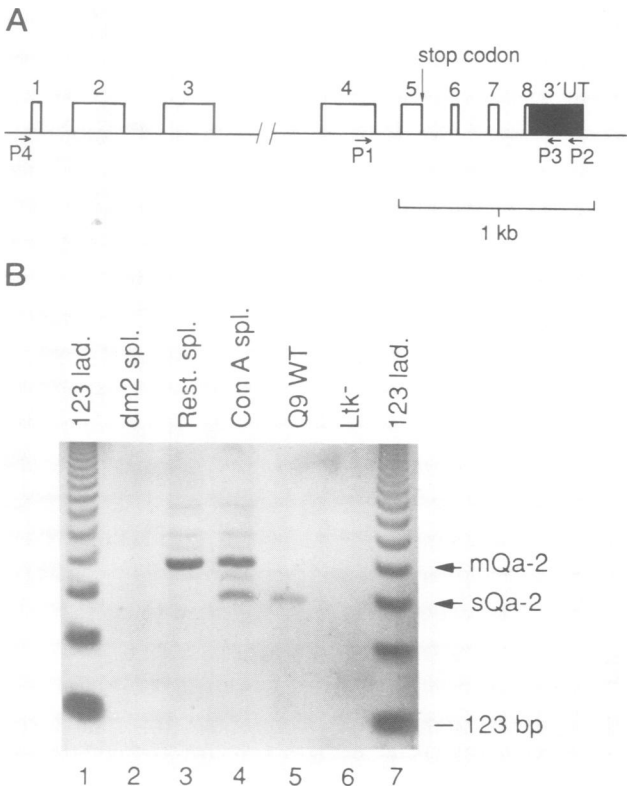

Fig. 3. PCR amplification of cDNA from several cell types identifies two forms of *Q7/Q9* transcripts. (**A**) Diagrammatic representation of the *Q7/Q9* gene with the locations and directions of the primers used for sequencing and PCR amplification. The *Q7/Q9* exons and 3′ untranslated region (3′ UT) are indicated by the open and closed boxes, respectively. (**B**) Two μg of total RNA from the indicated cells were used for the first strand cDNA synthesis. Thirty cycles of PCR amplification were carried out with the P1 and P2 primers on 1/10 of the cDNA reaction mix prepared from resting spleens of BALB/c Kh-H-2^{dm2} (lane 2), C57BL/10 (lane 3), Con A activated spleen of C57BL/10 (lane 4), Ltk$^-$ cells transfected with *Q9wt* gene (lane 5), and untransfected Ltk$^-$ cells (lane 6). Lanes 1 and 7 contain 123 bp ladder (BRL) as molecular size markers. *mQa-2* and *sQa-2* designations were given to the canonical and the novel *Q7/Q9* cDNA species, respectively. P3: 5′-GGGTCCAGGTGTGCAGGG.

Qa-2 (Flaherty *et al.*, 1985) score negative in this assay demonstrating the specificity of the primers and the conditions used for PCR (Figure 3b, lanes 2 and 6).

The *mQa-2* and *sQa-2* PCR products from Con A activated spleen cells were purified from low melting point agarose gel and sequenced using P_3 primer (described in Figure 3a). Figure 4 shows that *mQa-2* corresponds to the canonical Qa-2 cDNA encoding membrane bound form of the antigen (Steinmetz *et al.*, 1981; Waneck *et al.*, 1987). It contains exon 5 which determines all the Qa-2 features necessary for the PI processing, including ~21 amino acid hydrophobic domain followed by three charged residues. The shorter PCR product, *sQa-2*, lacks this exon. The partial sequences of these two forms of *Q7/Q9* cDNA and the C-termini predicted for their protein products are shown in Figure 4b. Excision of exon 5 in *sQa-2* removes the coding region for the hydrophobic domain and the short cytoplasmic tail as well as the stop codon of the mQa-2 cDNAs. This leads to the translation of 12 new residues encoded by exons 6 and 8 [exon 7 is absent in both *mQa-2* and *sQa-2* due to a non-functional splice acceptor site for this exon (Steinmetz *et al.*, 1981; Devlin *et al.*, 1986)]. The hydrophilicity of the deduced C-terminus product of *sQa-2* is consistent with the assumption that it encodes a secreted molecule.

Translation of the alternatively spliced forms of Qa-2 cDNAs leads to the expression of soluble and PI-linked Qa-2 molecules

To provide unequivocal evidence that alternatively spliced *Qa-2* transcripts lacking exon 5 can be translated into soluble Qa-2 molecules, we have cloned and expressed full length *sQa-2* and *mQa-2* cDNAs. Both forms of Qa-2 cDNAs were amplified from C57BL/10 Con A activated spleen RNA by

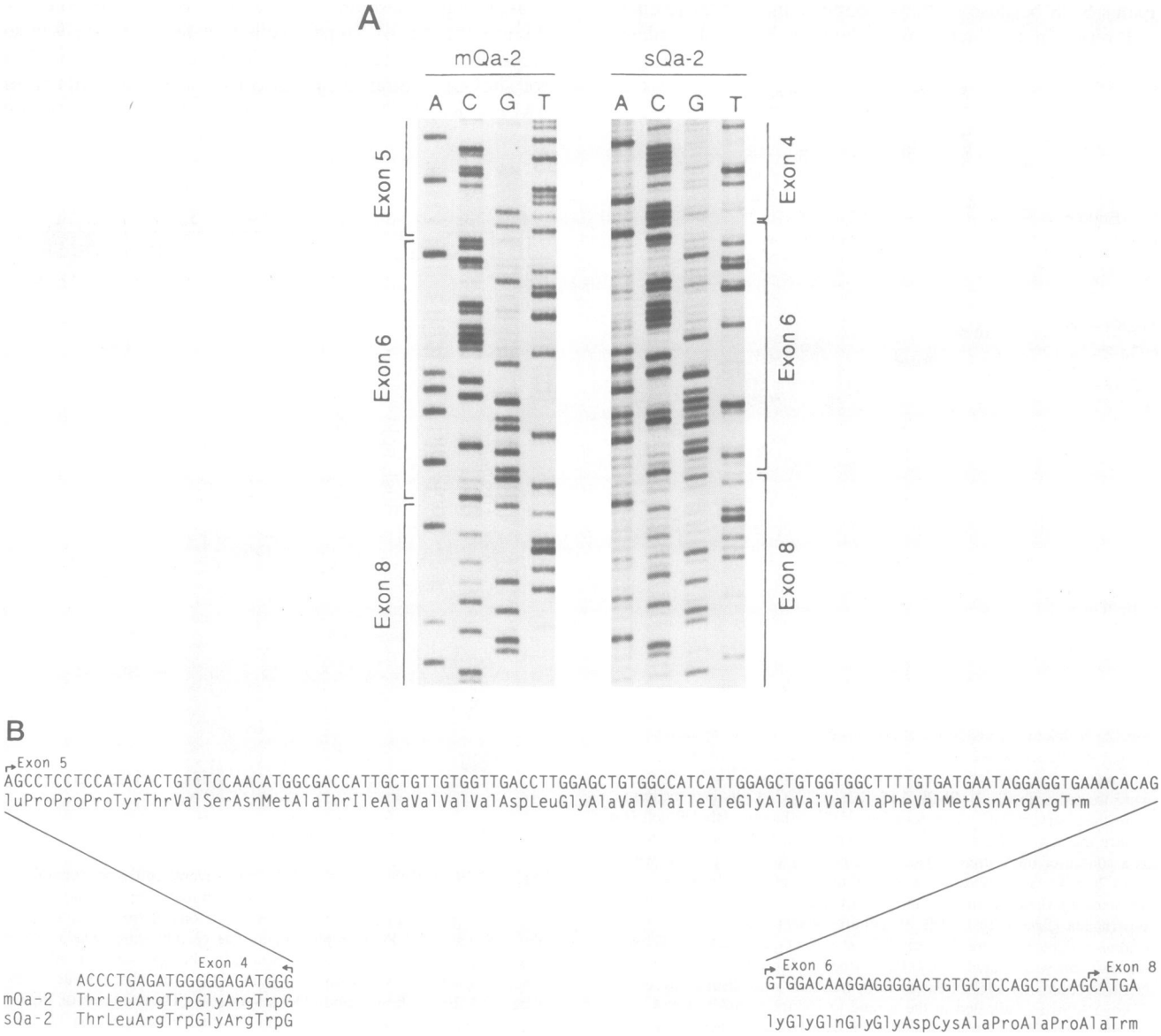

Fig. 4. *sQa-2* corresponds to a differentially spliced transcript of the *Q7/Q9* gene. (**A**) The DNA species designated as *mQa-2* and *sQa-2* in Figure 3 were purified from a low melting point agarose gel and used for sequencing by the dideoxynucleotide chain termination method. Sequencing was carried out with an antisense primer, P3, to the 3′ UT region of the *Q7/Q9* gene. (**B**) Nucleotide and deduced amino acid sequences of sQa-2 and mQa-2. Unlike the conanical *Q7/Q9* transcript, *mQa-2*, the alternatively spliced transcript, *sQa-2*, lacks exon 5 which encodes the putative transmembrane domain containing signals for the PI-modification of mQa-2 (Ulker *et al.*, submitted). The lack of exon 5 in sQa-2 results in an open reading frame for 12 hydrophilic residues encoded by exons 6 and 8. For the purpose of clarity, the common N-terminal sequences for both Qa-2 molecules are not shown.

PCR using primers derived from 5′ and 3′ untranslated regions of the *Q7/Q9* gene (see Materials and methods). The PCR products were fractionated on an agarose gel and DNA fragments with the predicted size of 1200−1400 bp were excised and cloned into a M13 vector. The full size *sQa-2* and *mQa-2* cDNAs with the expected restriction enzyme patterns were identified by sequencing of the DNA downstream from exon 4. The restriction fragments containing both types of cDNAs were then recloned into a eukaryotic expression vector, pcDNA1 containing a cytomegalovirus promoter and transcription termination/polyadenylation signals. Several cell lines were then transfected with the two forms of the cloned cDNAs and the cell surface expression of Qa-2 was analyzed by flow cytometry (FCM). While the Hepa-1 cells transfected with *mQa-2* cDNA express the PI-linked Qa-2 molecules on the surface (Figure 5b), the *sQa-2* transfectants scored negative (Figure 5a). To provide a direct evidence for the synthesis of the secreted product, transiently transfected COS-7 cells were metabolically labeled with [^{35}S]methionine for 10 h along with resting or Con A activated C57BL/10 spleen cells. The culture supernatants were analyzed by immunoprecipitation with Qa-2 specific antibodies. As shown in Figure 6, the mAb 20-8-4 precipitates ~39 kd molecules from the supernatants of the *sQa-2* transfectants only. These molecules are indistinguishable from sQa-2 secreted from C57BL/10 splenocytes (Figure 6, lanes 1 and 2). On longer exposures of this gel, the mAb 20-8-4 reactive material of approximate mol. wt of 39 kd can also be detected in lane 4 of Figure 6 (corresponding to the mQa-2 transfectants). We do not know whether this molecule is generated from mQa-2 by endogenous PI-PLC-like enzymes or if it is a PI processing intermediate or a degradation product of mQa-2 accompanying fortuitous cell death.

These data demonstrate clearly that sQa-2 molecules of predicted molecular weight are translated from novel, alternatively spliced Qa-2 transcripts. The enhanced secretion of sQa-2 in the activated T cells and in the transfected L cells is shown to correlate with the preferential transcription of Qa-2 mRNAs lacking exon 5.

Transcription of Qa-2 genes is widespread

Serologically detectable Qa-2 antigens were initially reported on adult mouse lymph nodes, spleen, thymus and on bone marrow cells (Flaherty, 1976). More sensitive methods such as cDNA cloning, S1 nuclease or RNase protection assays revealed the presence of *Qa-2* transcripts in other tissues including liver, kidney and whole embryos (Lalanne *et al.*, 1985; Fahrner *et al.*, 1987; Hedley *et al.*, 1989). The specificity of our primers (Figure 3b, lanes 2 and 6) and the tremendous sensitivity of the PCR technique over any other assay led us to reexamine the question of *Qa-2* tissue distribution and *sQa-2* expression in particular. Total RNAs isolated from different tissues of BALB/c J mice were analyzed by PCR. The results of the agarose gel fractionation of PCR products revealed the synthesis of *Qa-2* transcripts in all tissues examined (Figure 7a). However, the apparent level of expression (as judged by this semi-quantitative approach) varied among different tissues. We detected more *mQa-2* transcripts in mesenteric lymph node, spleen, thymus, liver, lung and kidney than in testes, heart and brain (Figure 7a). The expression was higher in day 12 and day 16 embryos than in day 18 embryos (Figure 7a, lanes 4−6). This is consistent with the Northern blot and S1 analysis data reported by Hedley *et al.* (1989). Interestingly, *sQa-2* transcripts were barely detectable in tissues expressing even large quantities of *mQa-2* (Figure 7a, lanes 7 and 8). No *sQa-2* was detected in tissues expressing low levels of *mQa-2*.

Since the presence of *mQa-2* transcripts in whole tissues could be attributed to contamination with passenger lymphocytes, several established cell lines of different tissue origins were also analyzed (Figure 7b). These included LL/2 (C57BL lung), TM3 (BALB/c Leydig cells of the testis), TM4 (BALB/c Sertoli cells of the testis), M-3 [(CXDBA)-F_1 melanoma], NOR 10 (C57BL/10 abdominal wall muscle, fibroblast-like) and RAG (BALB/c renal adenocarcinoma). While the steady state level of *mQa-2* and *sQa-2* transcripts was significantly higher in some cell lines, it was barely detectable in others (Figuer 7b, compare high expression in RAG with low level in M3). Where applicable,

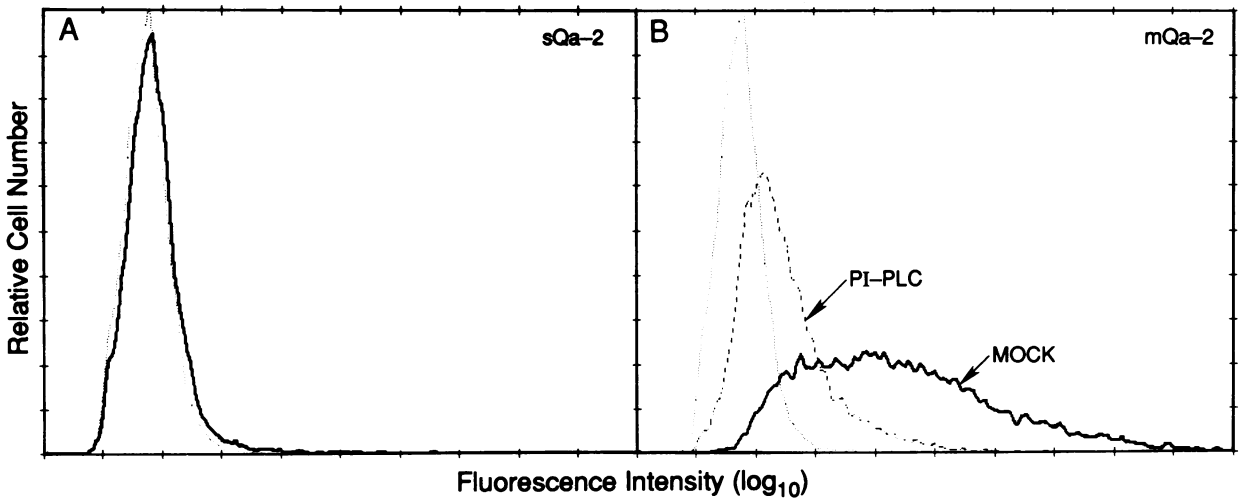

Fig. 5. The expression of cell surface Qa-2 antigen is detectable only in the cells transfected with *mQa-2* cDNA. Hepa-1 cells were stably transfected with *sQa-2* cDNA (panel A) or *mQa-2* cDNA (panel B) cloned in the eukaryotic expression vector pcDNAI. Transfectants were either treated with 0.1 U/ml PI-PLC for 1 h at 37°C (---) or left untreated (———) as indicated. Following incubation, cells were washed twice with PBS and then stained for flow cytometric analysis using Qa-2 diagnostic mAb 20-8-4 as a primary reagent and FITC-conjugated goat anti-mouse immunoglobulins antibody as a secondary reagent. Untransfected Hepa-1 cells were analyzed as controls (···).

a correlation was observed between the cell line and the tissue of origin in respect to the level of the *mQa-2* transcription. In addition, Southern blot analyses were carried out on PCR amplification products from these cell lines using either *mQa-2* or *sQa-2* specific oligos as probes. The BC_3HI (smooth muscle-like) cell line was used as a negative control since it was derived from C3H mouse which does not carry the genes for Qa-2 (Watts *et al.*, 1989). Southern blot hybridizations revealed the presence of both forms of the *Qa-2* transcripts in all the cell lines except BC_3HI (data not shown). Although there were some variations from cell line to cell line with respect to the total level of *s* and *mQa-2* transcription, the ratio of the steady state level of *sQa-2* versus *mQa-2* was similar to the ratio in the activated splenocytes, i.e. *sQa-2* represented ~40% of the total *Qa-2* transcripts.

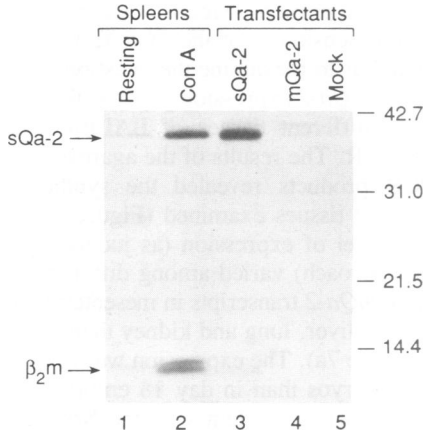

Fig. 6. Cells transfected with the *sQa-2* cDNA synthesize secreted Qa-2 molecule. COS-7 cells were transiently transfected with either *mQa-2* cDNA or *sQa-2* cDNA cloned in the eukaryotic expression vector pcDNAI. Seventy-two hours after transfection, cells were used for biochemical analysis. Resting (lane 1) or Con A activated C57BL/10 splenocytes (lane 2), *sQa-2* transfectants (lane 3), *mQa-2* transfectants (lane 4), and untransfected COS-7 cells (lane 5), were metabolically labeled with [^{35}S]methionine for 10 h. The culture supernatants were then harvested by centrifugation and used for immunoprecipitation with mAb 20-8-4. The source of the ~30 kd protein seen in resting splenocytes medium is not known (lane 1). Positions of mol. wt markers are indicated on the right. The weak signal of β_2m in lane 3 is due to the unfavorable association of simian β_2m with mouse class I molecules.

Discussion

Usage of exon 5 is regulated during T-cell activation

We have demonstrated here that the soluble ~39 kd Qa-2 molecule and the membrane PI-linked ~40 kd Qa-2 antigen are synthesized from two alternatively spliced transcripts of the same gene. The two mRNA species differ in the usage of exon 5. This exon is sufficient to encode all the features necessary for PI processing of Qa-2 cell surface precursor molecules (Ulker *et al.*, 1990) and is required for transduction of the T-cell activation signal via the Qa-2 antigen (Robinson *et al.*, 1989). Its removal leads to the translation of a truncated soluble Qa-2 molecule that is predicted to have a hydrophilic C-terminus. Interestingly, there is a marked preference for splicing out exon 5 in cultured L cells and in activated T cells. Stimulation of splenocytes by Con A results in an overall increase of Qa-2 production (Soloski *et al.*, 1986) and in a seletive shift in the proportion of *Qa-2* mRNAs lacking exon 5. The switching of the splicing mode is accompanied by an increase in the secretion of soluble Qa-2 molecules (Soloski *et al.*, 1986) and in the relative decrease of membrane bound antigens (Rabinowitz *et al.*, 1986). The molecular factors and mechanisms that contribute to the T-cell activation dependent splicing are presently unknown, but it is likely that they affect several genes simultaneously. Transcription of at least two other genes was

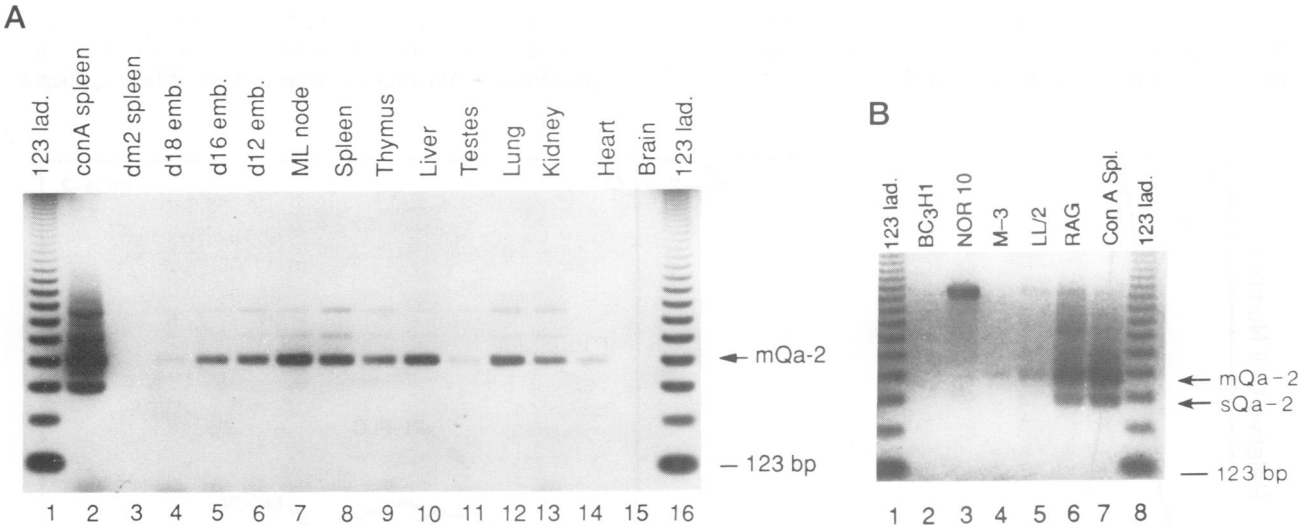

Fig. 7. The expression of *Q7/Q9* gene is ubiquitous. Total RNA was isolated from either tissues or established cell lines of different tissue origins. cDNA synthesis and PCR amplifications were carried out under the same conditions as described in the legend to Figure 3b. (**A**) The analysis of *Q7* expression in embryos and different tissues of the BALB/c mice. Equal volumes of the PCR reactions from the BALB/c embryos of the indicated stages (lanes 4–6) and the indicated tissues from adult animals (lanes 7–15) were analyzed on a 1.5% agarose gel and the DNA was visualized by EtBr staining. While activated spleen cells from the C57BL/10 (lane 2) were used as a positive control, spleen cells from BALB/c Kh-H-2^{dm2} strain (lane 3), which lacks the gene encoding Qa-2 antigen, were utilized as a negative control. *sQa-2* transcripts are not detectable under these conditions. (**B**) The analysis of *Q7/Q9* expression in established cell lines of different tissue origins. The indicated cell lines were analyzed under the same conditions as in (A). Lanes 1–8 correspond to: 1, molecular size marker; 2, smooth muscle-like cells derived from Qa-2 null C3H mouse strain; 3, fibroblast-like cells; 4, melanoma cells; 5, lung cells; 6, renal adenocarcinoma cells; 7, Con A-activated spleen cells; 8, as lane 1. Both forms of the Qa-2 transcripts are detectable in most of the cell lines analyzed.

reported to correlate with T-cell activation. These include CD45 (Birkeland *et al.*, 1989) and CD8 (Zamoyska *et al.*, 1985; Norment *et al.*, 1989). The phenotypic 'switching' of Qa-2 expression from a predominantly membrane bound form to a secreted form is somewhat reminiscent of immunoglobulin heavy chain (IgM) switching (Early *et al.*, 1980; Rogers *et al.*, 1980). During B-cell development, alternative processing of the immunoglobulin μ primary transcript results in regulated production of mRNAs encoding the secreted (μ_s) and membrane bound (μ_m) forms of IgM. In this case, however, the major factor determining the ratio of μ_s to μ_m is a poly(A) site choice rather than a splicing choice (Galli *et al.*, 1988). Since mQa-2 molecules are expressed on B cells, it would be of interest to study the Qa-2 splicing and the Qa-2 secretion during B-cell maturation and correlate it with events affecting IgM switching. Similarly, it was proposed for CD45 that B cells as well as T cells may change CD45 isotype expression during differentiation.

Excision of exon 5 represents only one of the several alternative splicing modes reported for class I genes. Many of the murine classical H-2 genes undergo differential splicing of exons 6, 7 and 8 (Lew *et al.*, 1986; McCluskey *et al.*, 1986; Rogers *et al.*, 1986). The biological significance and regulatory signals involved in these processes are not understood. In addition, *Q7* and *Q9* derived transcripts lacking exons 4–7 were identified in embryos, liver and transfected T cells (Fahrner *et al.*, 1987; Lalanne *et al.*, 1985; Waneck *et al.*, 1987) but are not known to be translated into proteins. Matsuura *et al.* (1990) reported two cDNAs derived from a fusion *Q8–Q9* gene which differ in usage of exon 5. Also, some cultured human cell lines secreted classical HLA molecules due to the aberrant splicing out of exon 5 encoding the transmembrane domain (Krangel, 1986). So far, this phenomenon has not been observed *in vivo* nor in the context of the non-classical human class I antigens.

Widespread expression of Qa-2 genes

The membrane form of Qa-2 antigen is serologically detectable on most lymphoid organs including spleen, thymus and lymph nodes (Flaherty, 1976). They are also abundant on primitive hemopoietic progenitors but are lost progressively upon differentiation (Bertoncello *et al.*, 1987). Most of the thymic and peripheral single positive $CD4^+CD8^-$ and $CD4^-CD8^+$ T cells express 10 times more Qa-2 molecules than do B cells (Tian and Soloski, 1989). In contrast, double positive $CD4^+CD8^+$ thymocytes appear negative by serological criteria (Rabinowitz *et al.*, 1986; Vernachio *et al.*, 1989). Recent expression analyses demonstrated that *Qa-2* genes are also transcribed in non-lymphoid tissues: lung, liver, kidney (Lalanne *et al.*, 1985) and early embryos (Fahrner *et al.*, 1987; Hedley *et al.*, 1989). We have confirmed these results using the PCR technique and showed that low level transcription of *Qa-2* genes occurs also in other tissues tested: heart, testes and brain, and in all tested cultured cell lines. Thus, it appears that the Qa-2 antigens have a much wider tissue distribution than originally thought, though the levels of expression predicted from the transcriptional activity of these genes are expected to be several magnitudes lower than the corresponding H-2 antigen levels. Interestingly, most tissues studied transcribe small amounts of *sQa-2* in addition to *mQa-2* RNAs. Our preliminary results suggest that the proportion of *sQa-2* is significantly enhanced in transformed tissue culture cell lines and is comparable with the ratio (~40%) of *sQa-2* to *mQa-2* observed in activated T cells.

Function of Qa-2 molecules

Although considerable information is now available concerning structure and expression of *Qa-2* genes and polypeptides, their functions remain unknown. Structural analyses of predicted Qa-2 proteins (Stroynowski, 1990) and demonstration of peptide binding by another Qa/Tla encoded class I product (Loveland *et al.*, 1990) suggest that these molecules may function in immune surveillance as antigen presenting structures. Unlike H-2 molecules, the Qa-2 polypeptides are non-polymorphic, and expressed at relatively low levels. Thus, it appears reasonable to propose that they can present only a limited set of non-polymorphic antigens having high affinity for Qa-2 class I structures. Several different antigen candidates have been considered in this regard: embryonal self-antigens which are absent during the T-cell tolerance induction stage but may aberrantly reappear on differentiating T cells or on tumors derived from other cells of the body; differentiation antigens necessary to drive the proliferation of some cell subsets; stress proteins marking cells for destruction; carbohydrate or other non-peptide antigens and antigens derived from non-viral pathogens (Asarnow *et al.*, 1988; Ostrand-Rosenberg *et al.*, 1989; Strominger, 1989; Stroynowski, 1990). Double negative $CD4^-CD8^-$ cytotoxic T cells expressing $\gamma\delta$ receptors were proposed as their potential ligands (Bluestone *et al.*, 1988; Ito *et al.*, 1989). What are the potential roles of the secreted Qa-2 molecules? Since PI-linked Qa-2 antigens can transduce T-cell activation signals and serve as targets for cytotoxic reactions, one can speculate that switching from the membrane bound to the secreted form may serve to make the host cell refractory to Qa-2 ligands and may contribute to the selective expansion or destruction of Qa-2 positive cells. Switching would decrease the number of cell surface targets participating in signal transduction or T-cell recognition and might additionally block their ligand receptors in solution. These hypotheses may now be tested directly as the cloning of cDNAs encoding sQa-2 and mQa-2 molecules makes it possible to overexpress these polypeptides and to study their ability to block T-cell activation signals and T-cell dependent killing.

Materials and methods

Animals

C57BL/10 and BALB/c mice were obtained from the Jackson Laboratory, Bar Harbor, ME. BALB/c Kh-H-2^{dm2} mice were bred in the animal facility at the California Institute of Technology.

Reagents and cell culture

$Q9^b$ constructs *Q9WT* and *Q9#3*, are derived from the *Q9* gene received from Dr Flavell's laboratory and were described elsewhere (Ulker *et al.*, 1990). The expression vector pcDNA I was purchased from Invitrogen, San Diego, CA. The Qa-2 diagnostic monoclonal antibody 20-8-4 (Sharrow *et al.*, 1984) was either purified on protein A–sepharose (Stroynowski *et al.*, 1987) or concentrated from hybridoma culture supernatants by passing through a Diaflo ultrafiltration membrane with a 100 kd molecular weight cutoff (Amicon, Danvers, MA). Normal rabbit serum was purchased from Cappel, West Chester, PA. Concanavalin A (Con A) was obtained from Sigma, St Louis, MO.

The thymidine kinase-negative L cells ($H\text{-}2^k$), hepatoma (Hepa-1, $H\text{-}2^b$) cells, *Q9WT* and *Q9#3* transfectants were described previously (Ulker *et al.*, 1990). Hepa-1 and L cell lines were maintained in α-minimum essential medium (α-MEM, Gibco Laboratories) containing 10% fetal calf serum,

2 mM L-glutamine and antibiotics. The transfectants were grown in the above medium supplemented with 400 μg/ml of the geneticine G418 (Gibco). Cell lines COS-7 (Gluzman *et al.*, 1981), BC_3HI (H-2^k), LL/2 (H-2^b), NOR 10 (H-2^b), RAG (H-2^d), TM3 and TM4 (H-2^d) were obtained from American Type Culture Collection (ATCC), Rockville, MA. All these cell lines were maintained in tissue culture under the conditions recommended by ATCC. The single cell suspensions of splenocytes are prepared by teasing mouse spleens into Hanks' balanced salt solution (Irvine Scientific, Santa Ana, CA). Cells were then washed three times with the same salt solution and passed through a nylon mesh to obtain single cells free of large tissue particles. The cell viability was assessed by trypan blue exclusion.

Activated T cells were obtained by culturing spleen cells for 72 h in RPMI 1640 medium containing 5% fetal calf serum, 50 μM 2-mercaptoethanol, 2 mM L-glutamine and antibiotics in the presence of 5 μg/ml of Con A.

cDNA synthesis and polymerase chain reaction

Total RNA was prepared from tissues and cell cultures as described by Chomczynski and Sacchi (1987). The first strand cDNA was prepared from 3 μg of RNA in a reaction volume of 20 μl using the Amersham cDNA synthesis kit according to the manufacturer's instructions. PCR was carried out in each case on 2 μl of the cDNA reaction mix added to 50 μl of total PCR reaction cocktail (Saiki *et al.*, 1988), consisting of 50 mM KCl, 10 mM Tris−Cl pH 8.3, 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μm dNTPs, 0.5 U of Taq polymerase (Cetus) and 500 pmol each of the amplification primers p_1 and P_2. P_1, 5′-ACCCTGAGATGGGGGAGATGG, is a sense oligo which corresponds to the 3′ end of the exon 4 encoding the α_3 domain of Qa-2, whereas P_2, 5′-CTTCGTGTGAAAGTATGGAGC, is an antisense oligo corresponding to the 3′ untranslated region of *Q7/Q9*. The reaction proceeded for 35 cycles in a PCR 1000 Thermal Cycler (Perkin-Elmer Cetus) programmed for 1 min at 94°C, 2 min at 64°C and 2 min at 74°C per cycle, followed by a final step of 7 min at 74°C. The PCR products were analyzed on a 1.5% agarose gel stained with ethidium bromide (EtBr).

DNA sequence analysis on the above PCR products was conducted as follows. One half of the PCR reaction mix was fractionated on a 1.5% low melting point agarose gel; gel was stained with EtBr and bands of amplified DNA were excised. DNA was then isolated by melting the agarose at 65°C and extracting it twice with phenol and once with chloroform:isoamylalcohol (24:1). DNA was then precipitated with ethanol and used for sequencing by the method of Zhang *et al.* (1988).

cDNA cloning

Total mRNA from Con A activated C57BL/10 splenocytes was used for the first strand cDNA synthesis. PCR was carried out on 2 μl of the cDNA reaction mix under the same conditions as described above, except that a pair of oligos—P2 and P4: 5′-CGGATCCGAGCAATGGCTCTAACA-ATGC were used. P4 primer is complementary to the 5′ end of the first *Q7/Q9* exon and contains a *Bam*HI site. P2 primer was also modified to include a *Bam*HI restriction enzyme site. The PCR products were fractionated on a 1.3% low melting agarose gel. DNA species corresponding to the expected sizes of *sQa-2* and *mQa-2* (~1.3 kbp and ~1.4 kbp, respectively) were excised from the gel and purified by phenol and chloroform extractions under the same conditions used for the purification of PCR products for sequencing. Purified DNA was then cleaved with *Bam*HI and ligated to *Bam*HI cut M13mp18 vector by standard DNA techniques (Sambrook *et al.*, 1989). This ligation mix was then used to transform competent DH5α F′ cells (BRL, Gaithersburg, MD). Plaque hybridization was carried out with *sQa-2* specific 5′-TTGTCCACCCCATCTC antisense oligomer (complementary to the junction of exons 4 and 6) and *mQa-2* specific 5′-ACAACAGCAATGGTCGCC antisense oligomer (complementary to exon 5) as described (Ulker *et al.*, 1990). Positive plaques were analyzed by dideoxy sequencing (Sanger *et al.*, 1977) to confirm the results obtained from plaque hybridizations. *sQa-2* and *mQa-2* were then excised from the M13mp18 vector and cloned into the *Bam*HI site of the eukaryotic expression vector pcDNAI.

DNA transfections

Transient transfections were carried out on COS-7 cells using Lipofectin Reagents (BRL, Gaithersburg, MD). Briefly, COS-7 monolayers in T75 flasks (~80% confluent) were washed thoroughly with serum-free Dulbecco's modified Eagle's medium (DMEM) (Gibco Laboratories) and refilled with 4 ml/flask of the same medium. DNA−lipofectin complex for each flask was prepared by mixing 50 μl of DNA (10 μg) with 50 μl of Lipofectin Reagents(30 μg in H_2O). The mixture was left on ice for 15 min and then added to the cells in T75 flasks dropwise. Monolayers were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 8 h, after which 4 ml of DMEM containing 20% fetal bovine serum were added to each flask. Monolayers were incubated for extra 72 h and cells were then harvested for biochemical analysis.

Stable tranfections with Lipofectin Reagents were carried out as described for transient transfections, except that cells were cotransfected with 500 ng of pSV_2neo plasmid and were grown in the presence of 400 μg/ml of G418.

Preparation of radiolabeled proteins

Labeling of cell surface proteins with ^{125}I was carried out by the lactoperoxidase method. Briefly, 1×10^7 cells, that had been washed three times with phosphate buffered saline (PBS) and resuspended in 150 μl of the same solution, were mixed with 50 μl of lactoperoxidase at 0.2 mg/ml (Sigma) and 1 mCi of carrier free Na ^{125}I (Amersham). The reaction was initiated by the addition of 10 μl of 1/1000 dilution of H_2O_2 (30%). After 5 min at 30−37°C, it was extended for an additional 5 min by the addition of another 10 μl of the same H_2O_2 dilution. The reaction was then stopped by washing the samples three times with PBS containing 10 mM KI.

Metabolic labeling of the cell cultures with [^{35}S]methionine was carried out as described previously (Stroynowski *et al.*, 1987). In brief, subconfluent monolayer cultures (T75 flasks) were washed twice with methionine-free maintenance medium and then incubated in the same medium supplemented with 5% dialyzed fetal calf serum and [^{35}S]methionine (Trans-label, ICN Radiochemicals, Irvine, CA) at ~150 μCi/ml for 10 h. Following radiolabeling, the culture medium was removed from the flasks and centrifuged at 150 000 *g* for 45 min to remove the insoluble material.

Phosphatidylinositol specific phospholipase C (PI-PLC) treatment of cells

The PI-PLC treatment of ^{125}I-labeled spleen cells or unlabeled transfectants was described elsewhere (Ulker *et al.*, 1990). In short, 1×10^7 cells were treated with 0.1 U/ml PI-PLC (American Radiolabeled Chemicals Inc., St Louis, MO) in 400 μl RPMI 1640 for 1 h at 37°C. Both cells and cell-free supernatants were harvested by centrifugation. Cells were then washed three times with PBS and used either for flow cytometric analysis or for the preparation of NP40 lysates.

The cell lysates were prepared by incubating the cell pellets in lysis buffer composed of 10 mM Tris−Cl, pH 7.5, 140 mM NaCl, 1.5 mM $MgCl_2$ and 0.65% NP40 (w/v) for 15 min on ice. Lysates were then sonicated for 20 s and the insoluble material was removed by spinning in an Eppendorf centrifuge for 2 min. Phenylmethanesulfonyl fluoride (PMSF) was added to a final concentration of 2 mM to all lysates and supernatants.

Flow cytometric (FCM) analysis

FCM analysis were carried out on 1×10^6 cells as follows. Subconfluent monolayers were harvested by trypsinization and washed twice with ice-cold PBS. Cells were then resuspended in 100 μl PBS containing a saturating concentration of mAb 20-8-4. After a 30 min incubation on ice, cells were washed three times with ice-cold PBS and incubated in 100 μl of 1:50 dilution of FITC-conjugated goat anti-mouse immunoglobulin for 15 min on ice. After washing three times with PBS, 1×10^4 cells were analyzed by a Flow Cytometer (Ortho Diagnostic Systems Inc., Model #2150).

Immunoprecipitation and SDS−PAGE

Immunoprecipitation of ^{125}I- and ^{35}S-labeled proteins were carried out as described previously (Ulker and Samuel, 1985) with the following modifications. NP40 lysates and culture medium were first precleared three times with normal rabbit serum (NRS) and Staph A and then precleared once with Staph A only. Samples were then incubated with a saturating concentration of mAb 20-8-4 for at least 4 h on ice. Immunocomplexes were precipitated with Staph A and washed three times with STN buffer (10 mM Tris−Cl, pH 7.5 containing 150 mM NaCl and 0.25% NP40). Subsequently, immunocomplexes were dissociated from Staph A by heating at 100°C for 3 min in 50 μl of electrophoresis sample buffer. Samples were then analyzed on a 5%/12% SDS−PAGE. Fluorography with En^3Hance (CIN Radiochemicals) was performed on ^{35}S-labeled protein gel according to the manufacturer's instructions. Autoradiography was carried out with Kodak XAR-5 film at −80°C.

Endo F treatment of radiolabeled proteins

Staph A precipitated immunocomplexes were resuspended in 40 μl of 100 mM Tris−Cl, pH 7.5 containing 0.5% SDS and 5% 2-mercaptoethanol. Samples were then incubated in a boiling water bath for 3 min to dissociate Staph A from immunocomplexes. After 2 min microcentrifugation, 8 μl of 10% Triton X-100 was added to each supernatant which was then divided into two equal fractions. While one fraction was left untreated, the other one was incubated with 2 μl of Endo-β-*N*-acetylglycosaminidase F (Boehringer Mannheim, 10 U/ml) for 1 h at 37°C. Subsequently, each

sample was mixed with 20 μl of 2× electrophoresis sample buffer and analyzed on a 5%/12% SDS–PAGE.

Acknowledgements

We thank K.Blackburn for excellent technical assistance and Drs D.Livant and J.Kobori for the critical review of the manuscript. This work was supported by NIH grant AI 17565.

References

Asarnow,D.M., Kuziel,W.A., Bonyhadi,M., Tigelaar,R.E., Tucker,P.W. and Allison,J.P. (1988) *Cell*, **55**, 837–847.
Bertoncello,I., Bartelmez,S.H., Bradley,T.R. and Hodgson,G.S. (1987) *J. Immunol.*, **139**, 1096–1103.
Birkeland,M.L., Johnson,P., Trowbridge,I.S. and Pure,E. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 6734–6738.
Bjorkman,P.J., Saper,M.A., Samraoui,B., Bennett,W.S., Strominger,J.L. and Wiley,D.C. (1987) *Nature*, **329**, 512–518.
Bluestone,J.A., Cron,R.Q., Cotterman,M., Houldon,B.A. and Matis,L.A. (1988) *J. Exp. Med.*, **168**, 1899–1916.
Chomczynski,P. and Sacchi,N. (1987) *Anal. Biochem.*, **162**, 156–159.
Devlin,J.J., Weiss,E.H., Paulson,M. and Flavell,R.A. (1985) *EMBO J.*, **4**, 3203–3207.
Early,P., Rogers,J., Davis,M., Calame,K., Bond,M., Wall,R. and Hood,L. (1980) *Cell*, **20**, 313–319.
Fahrner,K., Hogan,B.L.M. and Flavell,R.A. (1987) *EMBO J.*, **6**, 1265–1271.
Flaherty,L. (1976) *Immunogenetics*, **3**, 533–539.
Flaherty,L., DiBiase,K., Lynes,M.A., Seidman,J.G., Weinberger,O. and Rinchik,E.M. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 1503–1507.
Galli,G., Guise,J., Tucker,P.W. and Nevins,J.R. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 2439–2443.
Gluzman,Y. (1981) *Cell*, **23**, 175–182.
Hahn,A.B. and Soloski,M.J. (1989) *J. Immunol.*, **141**, 407–413.
Hedley,M.L., Drake,B.L., Head,J.R., Tucker,P.W. and Forman,J. (1989) *J. Immunol.*, **142**, 4046–4054.
Ito,K., Bonneville,M., Takagaki,Y., Nakanishi,N., Kanagawa,O., Krecko,E.G. and Tonegawa,S. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 631–635.
Krangel,M.S. (1986) *J. Exp. Med.*, **163**, 1173–1190.
Lalanne,J.-L., Transy,C., Guerin,S., Darche,S., Meulien,P. and Kourilsky,P. (1985) *Cell*, **41**, 469–478.
Lew,A.M., Maloy,W.L. and Coligan,J.E. (1986) *J. Immunol.*, **136**, 254–258.
Loveland,B., Wang,C.-R., Yonekawa,H., Hermel,E. and Fischer Lindahl,K. (1990) *Cell*, **60**, 971–980.
Matsuura,A., Schoss,R., Shen,F.W., Tung,J.-S., Hunt,S.W., Fischer,D.A., Hood,L.E. and Boyse,E.A. (1989) *Immunogenetics*, **30**, 156–161.
McCluskey,J., Boyd,L.F., Maloy,W.L., Coligan,J.E. and Margulies,D.H. (1986) *EMBO J.*, **5**, 2477–2483.
Norment,A.M., Lonberg,N., Lacy,E. and Littman,D.R. (1989) *J. Immunol.*, **142**, 3312–3319.
Ostrand-Rosenberg,S., Nickerson,D.A., Clements,V.K., Garcia,E.P., Lamouse-Smith,E., Hood,L. and Stroynowski,I. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 5084–5088.
Rabinowitz,R., Sharrow,S.O., Chatterjee-Das,S., Rogers,M.J. and Sachs,D.H. (1986) *Immunogenetics*, **24**, 391–401.
Robinson,P.J., Millrain,M., Antoniou,J., Simpson,E. and Mellor,A.L. (1989) *Nature*, **342**, 85–87.
Rogers,J., Early,P., Carter,C., Calame,K., Bond,M., Hood,L. and Wall,R. (1980) *Cell*, **20**, 303–312.
Rogers,M.J., Siwarski,D.F., Shacter,E., Maloy,W.L., Lillehoj,E.P. and Coligan,J.E. (1986) *J. Immunol.*, **137**, 3006–3012.
Saiki,R.K., Gelfand,D.H., Stoffel,S., Scharf,S.J., Higuchi,R., Horn,G.T., Mullis,K.B. and Erlich,H.A. (1988) *Science*, **239**, 487–491.
Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*. 2nd edn, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Sanger,F., Coulson,A.R., Barrell,B.G., Smith,A.J.H. and Roe,A. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463–5467.
Sharrow,S.O., Flaherty,L. and Sachs,D.H. (1984) *J. Exp. Med.*, **159**, 21–40.
Soloski,M., Vernachio,Y., Einhorn,G. and Lattimore,A. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 2949–2953.
Soloski,M.J., Hood,L. and Stroynowski,I. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 3100–3104.
Steinmetz,M., Moore,K.W., Frelinger,J.G., Sher,B.T., Shen,F.-W., Boyse,E.A. and Hood,L. (1981) *Cell*, **25**, 683–692.
Strominger,J.L. (1989) *Cell*, **57**, 895–898.
Stroynowski,I. (1990) *Annu. Rev. Immunol.*, **8**, 501–530.
Stroynowski,I., Soloski,M., Low,M. and Hood,L. (1987) *Cell*, **50**, 759–768.
Tian,H. and Soloski,M.J. (1989) *FASEB J.*, **3**, 1724.
Ulker,N. and Samuel,C.E. (1985) *J. Biol. Chem.*, **260**, 4319–4323.
Ulker,N., Hood,L.E. and Stroynowski,I. (1990) *J. Immunol.*, **145**, 2214–2219.
Vernachio,J., Li,M., Donnenberg,A.D. and Soloski,M.J. (1989) *J. Immunol.*, **142**, 48–56.
Waneck,G.L., Sherman,D.H., Calvin,S., Allen,H. and Flavell,R.A. (1987) *J. Exp. Med.*, **165**, 1358–1370.
Waneck,G.L., Sherman,D.H., Kincade,P.W., Low,M.G. and Flavell,R.A. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 577–581.
Watts,S., Davis,A.C., Gaut,B., Wheeler,C., Hill,L. and Goodenow,R.S. (1989) *EMBO J.*, **8**, 1749–1759.
Zamoyska,R., Vollmer,A.C., Sizer,K.C., Liaw,C.W. and Parnes,J.R. (1985) *Cell*, **43**, 153–163.
Zhang,H., Scholl,R., Browse,J. and Somerville,C. (1988) *Nucleic Acids Res.*, **16**, 1220.
Zinkernagel,R.M. and Doherty,P.C. (1980) *Adv. Immunol.*, **27**, 51–177.

Received on July 11, 1990