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Immunology

Qa gene expression: Biosynthesis and secretion of Qa-2 molecules in activated T cells

(regulation of *Qa* expression/T-cell activation/major histocompatibility complex)

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ABSTRACT **The biosynthesis and expression of the tissue-specific class I molecule Qa-2 have been studied in resting and activated T-cell populations. Polyclonal activation of T lymphocytes induces a 3- to 4-fold increase in the biosynthesis of Qa-2 molecules but no increase in cell-surface levels. Analysis of the biosynthetic pathway of the Qa-2 molecule in activated lymphocytes reveals that ≈70% of the newly synthesized Qa-2 molecules are secreted as soluble molecules. In resting-cell populations, Qa-2 remains entirely cell-associated. This process is unique to the Qa-2 molecule, since other class I molecules (e.g., H-2K^b and H-2D^b) synthesized by activated cells remain cell-associated. The possibility that the secreted Qa-2 molecule is the product of a new *Qa* gene or an alternatively spliced mRNA is considered. These results indicate that the Qa-2 molecules may not just function as a cell-surface recognition structure but also may serve a role as a soluble factor synthesized by activated lymphoid cell populations.**

The gene products of the major histocompatibility complex have been shown to serve critical roles in immune processes. It has been well appreciated that the class I molecules H-2K, H-2D, and H-2L and the class II molecules I-A and I-E both serve as potent stimulators of tissue graft rejection and function in T-cell recognition of foreign antigen (1). Recently, molecular genetic analysis has identified up to 35 distinct class I gene copies in the mouse genome (2, 3). The majority of these genes are located telomeric to the *H-2D,L* genes in the *Qa/Tla* region of murine chromosome 17 (3, 4). Serological and biochemical studies have demonstrated at least four distinct class I gene products encoded by this chromosomal segment (5, 6). These molecules have been designated Qa-1, Qa-2, Q10, and Tla, the thymus–leukemia antigen. Although all of these molecules share a similar subunit structure with the major transplantation antigens H-2K, H-2D, and H-2L, they differ considerably in their biological properties. For example, the Qa-2 molecules are nonpolymorphic and are expressed on selected subpopulations of bone marrow-derived cells (5). In contrast, H-2K, H-2D, and to a lesser extent H-2L molecules exhibit extensive polymorphism and ubiquitous tissue expression (7). The properties of Qa/Tla antigens together with the failure to demonstrate a role as a restriction element in T-cell recognition suggest that Qa/Tla molecules function in a novel manner in immune processes. An understanding of the biological function of Qa molecules is needed to complete our understanding of the biology of the MHC.

MATERIALS AND METHODS

Animals. Adult male C57BL/6 mice, age 6–10 wk, were utilized in this study. All were either purchased from The Jackson Laboratory or bred from our colony maintained at The Johns Hopkins University School of Medicine.

Serological Reagents. Antiserum reactive with Qa-2 was prepared by immunizing C57BL/6.K1 mice with C57BL/6 spleen and lymph node cells as described (8). Ascitic fluid containing the Qa-m2 monoclonal antibody was kindly provided by Ian McKenzie (Canberra, Australia) (9). Anti-H-2K^b antiserum was generated by immunizing (B10.D2 × A/J)F$_1$ mice with spleen and lymph node cells from B10.A-(5R) mice (10). Rabbit anti-mouse Ig and goat anti-mouse Ig were prepared as described (11). Fluorescein isothiocyanate-labeled affinity-purified rabbit antibody (Fab)$_2$ specific for the Fc portion of mouse γ chains and normal mouse serum (NMS) were purchased from Pel-Freez (12).

Isolation of Lymphoid Cell Subpopulation. Mouse spleens were teased into Hanks' balanced salt solution, and single-cell suspensions were prepared. Cell viability was assessed by trypan blue exclusion. Unactivated T cells were purified from spleen cell populations by panning on affinity-purified rabbit anti-mouse Ig-coated Petri dishes (13). Activated T cells were prepared by culturing spleen cells for 3 days in RPMI 1640 medium containing 5% fetal calf serum, 50 μM 2-mercaptoethanol, Con A at 5 μg/ml, glutamine, and antibiotics. Both resting and activated T cells prepared as described above were >90% Thy-1,2$^+$ Ig$^-$ as determined by flow cytometric analysis. In all cases where activated and resting cells were compared, animals were age- and sex-matched.

Immunofluorescence Analysis. Cells (2 × 10^6) were incubated with saturating amounts of the appropriate antibodies in 100 μl (total volume). NMS and H.11.4.1 (anti-H-2K^k) served as irrelevant control reagents for anti-Qa-2 antiserum (K1αB6) and the monoclonal antibody Qa-m2 and anti-H-2K^b reagents, respectively. After a 60-min incubation at 4°C, the cells were washed twice with phosphate-buffered saline containing 0.05% azide and 1% calf serum and were incubated with saturating amounts of fluorescein isothiocyanate-labeled (Fab)$_2$ rabbit anti-mouse γ chain for 30 min. For each analysis, 10,000 cells were analyzed for fluorescence with a fluorescence-activated cell sorter flow cytometer (FACS II, Becton Dickinson). Both fluorescence and forward scatter data were collected and analyzed as described (14).

Radiolabeling and Immunoprecipitation of Lymphoid Cell Populations. Lymphoid cell populations were radiolabeled at a density of 10^7 cells per ml in minimal essential medium (MEM) lacking methionine in the presence of 200 μCi (1 Ci = 37 GBq) of [^{35}S]methionine per ml (New England Nuclear). Pulse–chase studies were performed by preculturing cells at 10^7 per ml in MEM without methionine for 15 min at 37°C to deplete endogenous methionine pools. [^{35}S]Methionine was added to 200 μCi/ml and pulsed for 30 min. Cells were then washed once with prewarmed MEM containing 400 μCi of

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Abbreviations: NMS, normal mouse serum; NP-40, Nonidet P-40.

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methionine per ml, resuspended at 10^7 cells per ml, and harvested after various times (chase). Lactoperoxidase-catalyzed iodination of lymphoid cell surfaces has been described in detail (15). Radiolabeled cells were washed with Tris-buffered saline (TBS) (pH 7.4) and lysed with 0.5% Nonidet P-40 (NP-40) in the presence of protease inhibitors. The preclearing and immunoprecipitation protocols have been described in detail (10). Saturating amounts of appropriate antibodies were added, and immune complexes were either adsorbed to the protein A-bearing Cowan I strain of *Staphylococcus aureus* or precipitated by the addition of goat anti-mouse Ig. After the samples were washed with TBS containing 0.25% NP-40, 0.1% $NaDodSO_4$, and 0.2% deoxycholate and one time with TBS, they either were denatured and reduced for one-dimensional analysis or were prepared for two-dimensional analysis as described by O'Farrell (16) with the modifications suggested by Jones (15). After electrophoresis, gels were stained with Coomassie blue and extensively destained. Radioactivity was detected by fluorography. Molecular weights were estimated with molecular weight markers (Bio-Rad) that were run in parallel.

Differential Centrifugation Analysis. Cell-free medium (supernatant from centrifugation at 10,000 × *g*) was subjected to centrifugation at 160,000 × *g* for 60 min at 4°C. The supernatant was removed, and the pellet was solubilized with NP-40 for 12 hr at 4°C, and both the supernatant and the solubilized pellet were immunoprecipitated and analyzed by two-dimensional gel electrophoresis as described above.

RESULTS

Biosynthesis and Expression of Qa-2 Molecules in Resting and Activated T-Cell Populations. Since the major peripheral lymphoid cells that express the Qa molecules are T cells (5), we determined whether the biosynthesis and cell-surface expression of Qa-2 changed as a consequence of T-cell activation. An analysis of cell-surface levels of H-$2K^b$ and Qa-2 on resting and activated T cells is shown in Fig. 1. Both resting and activated T-cell populations were 85–95% Qa-2^+. Comparison of the levels of H-$2K^b$ expression showed an approximate 4-fold increase in surface mean fluorescence intensity (Fig. 1 *Top*). In contrast, when only the Qa-2^+ cells were considered, the levels of Qa-2 in resting versus activated cells showed little difference (Fig. 1 *Middle* and *Bottom*). Examination of the forward scatter for both resting and activated T cells demonstrated an ≈1.4-fold increase in mean forward-scatter channel number (data not shown). Thus, any small increase in Qa-2 surface expression is likely to be due to the increase in cell size occurring during activation.

In parallel with the cell-surface immunofluorescence studies, equal numbers of resting and activated T cells were radiolabeled with [^{35}S]methionine for 4 hr. Cell lysates were prepared, immunoprecipitated, and analyzed by $NaDodSO_4$/PAGE. Fig. 2 depicts a densitometric analysis of the Qa-2 and H-$2K^b$ antigens recovered from resting and activated T-cell populations. When compared to resting cells, activated T cells synthesized 4- to 5-fold more Qa-2. A similar increase was observed for the H-$2K^b$ class I molecule. The results above demonstrate that activated T cells synthesize increased levels of molecules reactive with anti-Qa-2 antiserum. However, this increase in synthesis is not reflected in an increase in cell-surface expression.

Qa-2 Molecules Synthesized by Resting and Activated T Lymphocytes. The observation described above suggested that a fundamental difference exists in the biosynthetic pathway for Qa-2 molecules in resting and activated cells. It has been reported that a class I molecule, Q10, is synthesized and secreted in the liver (6). In the light of this information, we examined by two-dimensional gel electrophoresis the molecular species reactive with anti-Qa-2 antiserum that

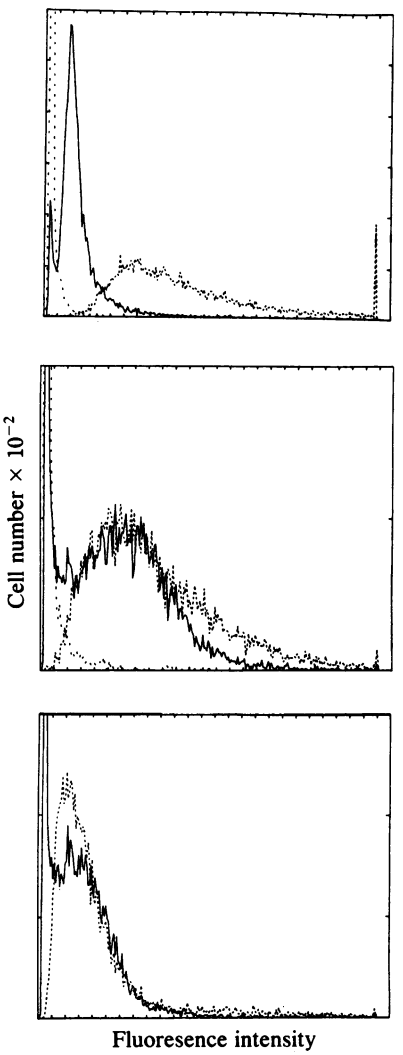

FIG. 1. Class I molecule expression on resting and activated T-cell populations. Fluorescence histograms of resting (—) and activated (----) T lymphocytes treated with anti-H-$2K^b$ antiserum (*Top*), anti-Qa-2 antiserum (*Middle*) and monoclonal antibody Qa-m2 (*Bottom*). Control reagents are NMS (– –, *Middle*) and H.11.4.1 (– –, *Top*). Fluorescein isothiocyanate-conjugated rabbit anti-mouse γ chain was used as a secondary reagent.

were synthesized and/or secreted by resting and activated lymphocytes. Spleen cells or mitogen-stimulated cells were continuously labeled for 6 hr with [^{35}S]methionine, and both cells and medium were harvested. The cells were lysed in NP-40, and both cytoplasmic extracts and medium were immunoprecipitated with anti-Qa-2 antiserum. Two-dimensional gel electrophoresis of the immunoprecipitates from resting spleen cells is shown in Fig. 3 *A* and *C*. Also included in Fig. 3 is an analysis of the Qa-2 molecules expressed on the cell surface of spleen cells as detected by lactoperoxidase-catalyzed iodination (Fig. 3*E*). The results demonstrate that during the 6-hr labeling period, two major species reacting with anti-Qa-2 antiserum were synthesized—a 38-kDa species and a more acidic 40-kDa species (Fig. 3*A*). Under the conditions of labeling, no Qa-2 was found in the medium (Fig. 3*C*). Comparison of cell-surface and biosynthetically labeled Qa-2 revealed that the 40-kDa species is identical in charge and molecular weight to the mature cell-surface form (compare *A* and *E* in Fig. 3). The 38-kDa polypeptide detected by biosynthetic labeling is most likely an intracellular precursor of the mature cell-surface form. Thus, in resting lymphocytes, the molecules reactive with anti-Qa-2 antiserum are

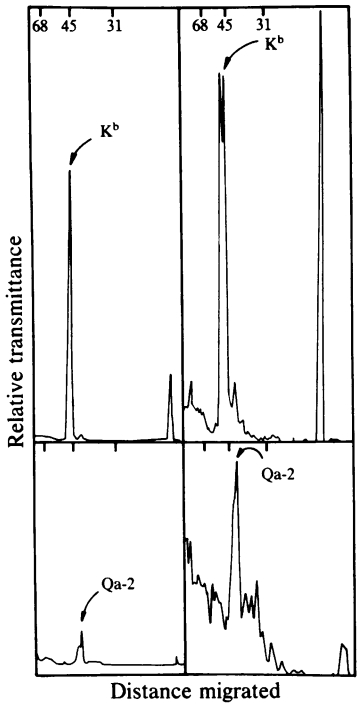

FIG. 2. Qa-2 biosynthesis in resting (*Left*) and activated (*Right*) T-cell populations. Resting or activated T lymphocytes were continuously radiolabeled with [^{35}S]methionine for 4 hr and lysed in NP-40, and cytoplasmic extracts were immunoprecipitated with anti-H-2K^b (*Upper*) or anti-Qa-2 (*Lower*) antiserum and analyzed by NaDodSO$_4$/PAGE. Displayed are densitometric scans of relevant lanes. Each analysis represents 10^7 cell equivalents. The positions of molecular weight markers are indicated, and the specific species recognized by anti-H-2K^b (K^b) or anti-Qa-2 (Qa-2) antiserum are designated with arrows.

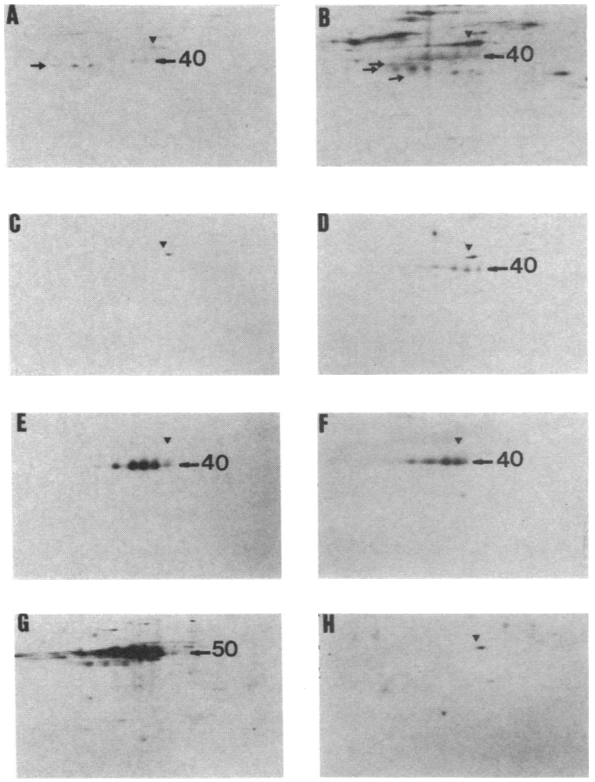

FIG. 3. Analysis of Qa-2 molecules synthesized and secreted by resting and activated lymphoid cells. Both total spleen and activated T cells were continuously radiolabeled with [^{35}S]methionine for 6 hr or vectorally labeled by lactoperoxidase-catalyzed iodination. Cell lysates or cell-free medium was subjected to immunoprecipitation with anti-Qa-2 (*A–F*) or anti-H-2K^b (*G* and *H*) antiserum. Shown are Qa-2 immunoprecipitates from [^{35}S]methionine-labeled spleen cell lysates (*A*) and media (*C*), activated T-cell lysates (*B*) and media (*D*), immunoprecipitates from ^{125}I-labeled spleen (*E*) and activated T cell (*F*), and anti-H-2K^b immunoprecipitates from activated T-cell lysates (*G*) and cell-free medium (*H*). Each panel represents an analysis of 10^7 cell equivalents. The positions and kilodaltons of species specifically precipitated by anti-Qa-2 or anti-H-2K^b antiserum are indicated by arrows. All other spots are found in NMS precipitates. The position of a standard spot detected by Coomassie blue staining is indicated by the inverted triangle. The basic end of the gels is on the left and the acidic end is on the right. Equivalent portions of each autoradiogram are presented.

synthesized as a low molecular weight precursor, which is processed to a higher molecular weight form that remains cell-associated, presumably as an integral membrane protein.

Two-dimensional analysis of the Qa-2 species synthesized by activated T cells displayed a more complex pattern (Fig. 3*B*). Three low molecular weight species (see arrows in Fig. 3) were observed, a major species of 35 kDa and two minor species of 34 and 38 kDa. The 38-kDa species appeared to be identical to that observed in resting cells, while the 34-kDa and 35-kDa species were unique to activated cells. Also found cell-associated was a heterogeneous 40-kDa species. Interestingly, a 40-kDa species was detected in the medium by anti-Qa-2 antiserum (Fig. 3*D*). Moreover, the Qa-2 molecule found in the medium had a more acidic isoelectric point distribution than did the cell-associated 40-kDa molecule. The secreted 40-kDa Qa-2 molecule was found to be associated with β_2-microglobulin (data not shown). Also included in this analysis is a profile of the Qa-2 species detected on the surface of activated T lymphocytes (Fig. 3*F*). Both the 40-kDa cell-surface molecule and the 40-kDa molecule found to be cell-associated during biosynthetic labeling have similar isoelectric point distributions. In addition, both cell lysates and cell-free medium from activated T cells were immunoprecipitated with anti-H-2K^b (Fig. 3 *G* and *H*) or anti-H-2D^b (data not shown) antisera and were analyzed. All of the H-2K^b and H-2D^b synthesized by activated T cells remained cell-associated. Thus, molecules reactive with anti-Qa-2 antisera are synthesized and selectively secreted by activated T lymphocytes. Other class I molecules (e.g., H-2K^b and H-2D^b) remain cell-associated under the labeling conditions used.

To determine the relationship between the various biosynthetic forms and to evaluate the proportion of newly synthesized Qa-2 that remains cell-associated and/or is secreted in activated T lymphocytes, pulse–chase studies were performed. Activated T cells were pulsed for 30 min with [^{35}S]methionine and chased in medium containing excess unlabeled methionine for various periods of time. Analysis of Qa-2 molecules in both cytoplasmic extracts and cell-free medium is shown in Fig. 4. Within 1 hr after the pulse, the predominant radiolabeled species were the 34-, 35-, and 38-kDa forms, with a small amount of label associated with the 40-kDa cell-associated species. Three hours after pulse labeling, the radioactivity associated with the 34-, 35-, and 38-kDa forms decreased, and an increase in both cell-associated and secreted 40-kDa species was seen. Little change in cell-associated versus secreted 40-kDa species was seen at 6 hr of chase. It should be noted that the 40-kDa secreted Qa-2 molecule had a slightly more acidic isoelectric distribution than did the 40-kDa species remaining cell-associated (Fig. 4 *E* and *F*). These results are consistent with a biosynthetic pathway for Qa-2 in activated cell populations in which the 34-, 35-, and 38-kDa intracellular precursors are processed to 40-kDa forms. The 40-kDa molecules then either remain cell-associated or are secreted. In these studies, from

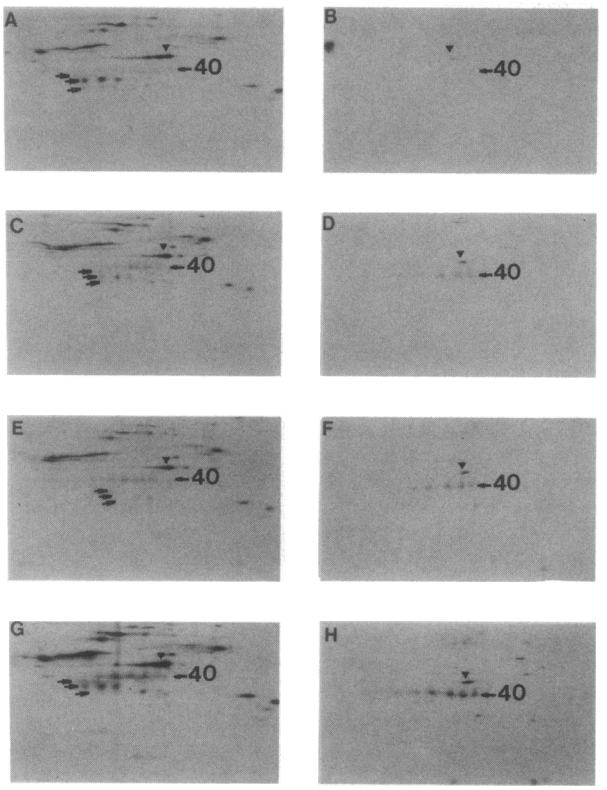

FIG. 4. Pulse–chase analysis of Qa-2 biosynthesis and secretion. Activated T lymphocytes were pulsed with [^{35}S]methionine, followed by a nonradioactive chase. Depicted are Qa-2 immunoprecipitates from a 1-hr chase, cell-associated (*A*) and medium (*B*); from a 3-hr chase, cell-associated (*C*) and medium (*D*); and from a 6-hr chase, cell-associated (*E*) and medium (*F*). Also depicted are Qa-2 immunoprecipitates from cell lysates (*G*) and medium (*H*) derived from activated T cells continuously labeled in parallel. Each analysis represents 10^7 cell equivalents. The species (and its mass in kilodaltons) specifically recognized by anti-Qa-2 antiserum is marked with arrows. The position of a standard spot detected by Coomassie blue staining is indicated by the inverted triangle. The basic end of the gel is on the left, and the acidic end is on the right. Equivalent portions of each autoradiogram are presented.

≈70% of the radiolabeled Qa-2 can be chased into the secreted form with ≈30% remaining cell-associated.

Qa-2 Is Secreted as a Soluble Molecule. Several investigators have reported the synthesis and secretion of class I molecules by lymphoid and myeloid cell populations as membrane vesicle-bound structures (17–19). Cell-free medium from continuously labeled activated T cells was centrifuged at 160,000 × *g* for 60 min. The supernatant was removed, and the pellet was solubilized in NP-40. Both supernatant and solubilized pellet were immunoprecipitated with anti-Qa-2 antiserum and analyzed. All of the secreted Qa-2 was recovered in the 160,000 × *g* supernatant (Fig. 5). Thus, the Qa-2 molecules synthesized and secreted by activated lymphocytes are not associated with membrane-bound vesicles but, instead, are secreted as a soluble protein.

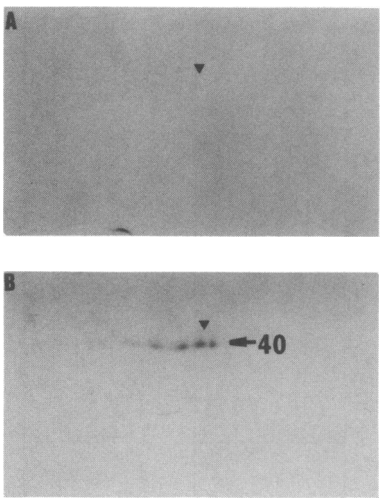

FIG. 5. Secreted Qa-2 is a soluble molecule. Activated T lymphocytes were continuously labeled with [^{35}S]methionine for 6 hr. The cell-free medium was centrifuged at 160,000 × *g* for 60 min. The supernatant was removed, and the pellet was solubilized with detergent. Both detergent-solubilized pellet (*A*) and supernatant (*B*) were immunoprecipitated with anti-Qa-2 antiserum and analyzed by two-dimensional gel electrophoresis. Equivalent portions of each autoradiogram are shown. The arrow points to the 40-kDa Qa-2 species. The basic end of the gel is on the left, and the acidic end is on the right. The position of a standard spot detected by Coomassie blue staining is indicated by the inverted triangle.

DISCUSSION

The studies reported above demonstrate that the biosynthesis of Qa-2 molecules is increased several-fold after T lymphocyte activation. However, this increase in synthesis is not reflected by a corresponding increase in cell-surface levels. Further study has shown that a 40-kDa molecule reactive with Qa-2 antiserum is synthesized and secreted as a soluble molecule by activated cells. In contrast, resting lymphocytes synthesize Qa-2 molecules, which remain cell-associated.

The secretion of Qa-2 molecules may explain the failure to detect new gene products on the surface of mouse L cells transfected with *Qa* genes (20–22). Expression of the BALB/c *Q6* gene could only be observed by utilizing a hybrid gene in which the 5′ end was derived from the *Q6* gene and the 3′ end was derived from *H-2L^d* (20). The presence of tissue-specific regulatory sequences in the 3′ region of the *Q6* gene but not the *H-2L^d* gene was suggested (20, 22). Recently, the transfection of the *Q6*, *Q7*, *Q8*, and *Q9* genes from B10 mice has been reported (21). In this case, the presence of new polypeptides reactive with anti-Qa-2 antiserum was observed in the cytoplasm of transfected cells but not on the cell surface. Based on our observation that molecules reactive with anti-Qa-2 antisera are secreted by activated lymphocytes, we would suggest that the failure to detect new protein products on the surface of transfected cells may, in some cases, be due to the production of a secreted protein product.

Activated T lymphocytes synthesize 40-kDa Qa-2 molecules, which either remain cell-associated or are secreted. Several mechanisms could account for the synthesis of cell-associated and secreted Qa-2 molecules. First, the secreted Qa-2 could represent a proteolytic product in which newly synthesized molecules, either during transport from the Golgi or upon reaching the cell surface, are cleaved adjacent to the cell membrane. In this manner, a soluble molecule lacking the hydrophobic transmembrane segment would be released. The observations that there is no detectable reduction in molecular weight, that Qa-2 molecules are readily found on the surface of activated T cells, and that no other class I molecules are released from the cell weigh strongly against this possibility. A second possibility is that the Qa-2 molecule is not an integral membrane protein but exists as an extrinsic protein on the cell surface, associated with another cell surface component that anchors it to the membrane. After activation, changes in either the anchoring structure or the Qa-2 molecule could cause newly synthesized Qa-2 to be transported extracellularly. Evidence against this model is that cell-surface Qa-2 molecules can be found associated with cytoskeletal elements (23) and are

radiolabeled with TID, a lipid-soluble photoactivatable probe (G.E. and M.J.S., unpublished data). A third possibility evokes an RNA splicing mechanism in which Qa-2 transcripts synthesized after activation utilize an alternative splice site, generating a mRNA species encoding a molecule with a unique COOH terminus that lacks a transmembrane segment. Studies on the structure of class I genes and transcripts have documented that alternative splicing, involving either the 5′ or 3′ exons, will generate a mRNA encoding a distinct class I molecule with unique NH_2 or COOH termini (24–26). Thus, alternative splicing is an attractive possibility. The last possibility is that another *Qa* gene product, detectable with anti-Qa-2 antisera, is synthesized in activated T cells. Recently, the products of the transfected *Q6*, *Q7*, *Q8*, and *Q9* genes were all found to be recognized by anti-Qa-2 antiserum (21). Thus, the *Qa* chromosomal region contains a number of class I genes that encode molecules that are serologically related. At present, we favor the last two possibilities but cannot distinguish between the two. Clearly, analysis at the protein and nucleic acid levels will be required before a definitive conclusion can be reached.

The existence of soluble class I molecules has been reported previously (27–29). These studies described a 40-kDa protein associated with β-2 microglobulin that exists as a high molecular weight, multimolecular complex in serum (27–30). In addition, the 60-kDa form (heavy chain + β_2-microglobulin) of serum class I molecules was identified and considered to be a proteolytic fragment of class I cell-surface molecules, since at least some of these molecules reacted with anti-H-2K and anti-H-2D alloantiserum and had a molecular mass (≈37–40 kDa) similar to papain-solubilized H-2K/D molecules (28, 29). However, based on the results presented above and the observation that anti-H-2K or anti-H-2D reagents crossreact with *Qa* subregion-encoded molecules (31–33), the possibility that Qa-2 molecules may be a component of either the high or low molecular weight serum class I structures needs to be considered. Recently, the *Qa* region gene *Q10* has been shown to encode a soluble class I molecule that is synthesized in the liver and found in serum (6). This 40-kDa molecule is distinct from Qa-2 since an anti-peptide reagent specific for Q10 does not react with secreted Qa-2 molecules nor does anti-Qa-2 antiserum react with the product secreted by L cells transfected with the *Q10* gene (A. Leu, personal communication). Thus, it appears that several soluble class I molecules may exist in serum derived from liver and perhaps lymphoid tissue.

We have demonstrated that only after activation do T cells synthesize a secreted molecule reactive with anti-Qa-2 antiserum. Pulse-chase studies revealed that 70% of molecules reactive with anti-Qa-2 antiserum are secreted, while 30% remains cell-associated. It is not known whether all cells or a distinct subpopulation produces the cell-associated and/or secreted forms. It is intriguing to speculate that functionally distinct T-cell subpopulations may synthesize the different forms. Although the function of the Qa-2 molecule is not known, the recent observation that *in vivo* administration of anti-Qa-2 monoclonal antibodies down-regulated T-cell responses suggests a role in immune function (34). It has been proposed that secreted class I molecules may regulate T-cell function either by blocking cytotoxic T-cell recognition or by inducing a tolerant state (35). Possibly, Qa-2 molecules may serve dual roles both as cell-surface structures and as soluble effector molecules produced by activated lymphocytes. Studies aimed at understanding the events that trigger the secretion of Qa-2 and the identification of the cells that bind secreted Qa-2 may shed light on a functional role(s) for Qa-2 molecules.

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