Transporters Associated with Antigen Processing (TAP)-independent Presentation of Soluble Insulin to α/β T Cells by the Class Ib Gene Product, Qa-1b

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Summary

T cell hybridomas isolated from nonresponder H-2b mice immunized with pork insulin were stimulated by insulin in the presence of major histocompatibility complex (MHC)-unmatched antigen presenting cells. The restriction element used by these CD4+ T cells was mapped to an oligomorphic MHC class Ib protein encoded in the T region and identified as Qa-1b using transfectants. The antigenic determinant was localized to the insulin B chain, and experiments with truncated peptides suggested that it is unexpectedly long, comprising most or all of the 30 amino acid B chain. The antigen processing pathway used to present insulin to the Qa-1b–restricted T cells does not require transporters associated with antigen processing (TAP), and it is inhibited by chloroquine. A wide variety of cell lines from different tissues efficiently present soluble insulin to Qa-1b–restricted T cells, and insulin presentation is not enhanced by phagocytic stimuli. Our results demonstrate that Qa-1b can function to present exogenous protein to T cells in a manner similar to MHC class II molecules. Therefore, this class Ib protein may have access to a novel antigen processing pathway that is not available to class Ia molecules.

Key words: antigen processing • major histocompatibility proteins • class Ib molecules • T cells • insulin

The family of MHC class I–related molecules in mice includes the “classical” (class Ia) proteins H-2K, D, and L, “nonclassical” (class Ib) molecules encoded in the Q, T, and M regions, and β2-microglobulin (β2m)-associated molecules such as CD1 that are encoded by genes not linked to the MHC (1–3). In most species, the class Ib genes significantly outnumber the class Ia genes but the proportion of class Ib pseudogenes is relatively high. Compared with class Ia genes, class Ib genes are relatively non-polymorphic, with few alleles and relatively small differences between alleles. The tissue distribution of class Ib gene products is heterogeneous, and expression levels are generally lower than those observed for class Ia proteins. Although the function of class Ib molecules remains unknown, it is likely that different molecules may have different functions, and there is a growing body of evidence that certain nonclassical class I proteins have specialized antigen presentation function. For example, H-2M3 has a specialized capacity to present N-formylated peptides which, regardless of sequence, bind poorly to class Ia molecules (4). This protein may have evolved to play a special role in the immune response to bacteria (5). Human CD1b is selectively targeted to endosomal compartments (6), where it can bind and present bacterial lipid antigens to TCR α/β T cells (7, 8).

The T region–encoded Qa-1 molecule, first identified serologically (9), has long been considered as a transplantation antigen that can be recognized by alloreactive CTLs (10–13). Qa-1 is expressed in tissues with a distribution similar to class Ia proteins but cell-surface density is relatively low. Four Qa-1 alleles have been characterized by serological and CTL assays, and the genes encoding two of these, Qa-1b (14, 15) and Qa-1a (16), have been cloned. A large fraction of Qa-1b–reactive TCR α/β CTLs have been shown to recognize a nonameric peptide, Q dm (Qa-1 determinant modifier), derived from the signal sequence of...
class Ia H-2D and L molecules (17, 18). Peptide extraction studies suggest that the peptides bound to Qa-1a molecules in activated T cells and transfected cells have limited heterogeneity, and that Qdm is the dominant peptide associated with this protein (18–20). Additional studies suggest that other antigens may also be presented by Qa-1a. Vidovic et al. (21) reported the isolation of a Qa-1b-restricted TCR γ/δ T cell hybridoma that recognizes the amino acid polymer poly(Glu10Tyr60). Imani and Soloski (22) demonstrated that cell surface Qa-1a expression is selectively upregulated after heat shock. A tryptic digest of M. bovis bovis 65-kD heat shock protein was observed to stabilize cell surface Qa-1a molecules, suggesting that this protein may be involved in presenting heat shock protein-derived peptides to the immune system. Jiang et al. (23) reported that Qa-


t-1a-restricted CTLs are involved in peripheral deletion of TCR Vβ8CD4+ T cells after injection with staphylococcal enterotoxin B (SEB) superantigen. These CTLs killed TCR Vβ8- but not Vβ8+ T cells, suggesting that they recognize an endogenous TCR Vβ-derived peptide(s) presented by Qa-1a (23). Bouwer et al. (24) demonstrated recently that Qa-1a-restricted CTLs are induced after infection with the intracellular pathogen, Listeria monocytogenes. These CTLs specifically lyse Qa-1a-expressing L. monocytogenes-infected cell lines, indicating that they directly recognize a bacterial antigen presented by Qa-1a. Thus, it is clear that Qa-1a can function as an antigen-presenting molecule in a variety of situations, but generalizations concerning its role in the immune system are difficult to make.

In this study, we describe T cell hybridomas isolated from nonresponder H-2d mice immunized with pork insulin that recognize an insulin B chain determinant presented by Qa-1a. The antigen processing pathway used to present insulin to these T cells appears to be distinct from the unconventional pathway(s) described previously for the presentation of exogenous protein antigens to MHC class Ia-restricted T cells.

Materials and Methods

Protein and Peptide Antigens. Beef and pork insulin were purchased from Elanco Animal Health (Indianapolis, IN). The desoctapeptide of beef insulin and sulfonated B chain were generated as described previously (25). Sperm whale myoglobin was purchased from Sigma Chemical Co. (St. Louis, MO). Peptides were synthesized using a peptide synthesizer (model 430A; Applied Biosystems Inc., Foster City, CA) in the Emory University Microchemical Facility or by using F-MOC chemistry with a Symphony multiple peptide synthesizer (Rainin Instrument Co., Inc., Woburn, MA) in the Emory University Microchemical Facility or by using F-MOC chemistry with a Symphony multiple peptide synthesizer (Rainin Instrument Co., Inc., Woburn, MA).

Mice. Female C57BL/10SnJ (B10), C57BL/6J (B6), B10. BR/SgSnJ, C3H/HeJ, BALB/cByJ, A/J, A.BY/SnJ, and SJL/J were purchased from The Jackson Laboratory (Bar Harbor, ME). B6-Tlaa and A-Tlaa mice were maintained under pathogen-free conditions at Johns Hopkins School of Medicine. Mice were used at 8–11 wk of age.

Cells and Cell Lines. The Qa-1b-expressing cell transfectant, L-g37, and vector control, L-V, were generated as described (22). TH2.2 (29) and LB 27.4 (30) are H-2d-d B hybridomas, whereas A20 is a BALB/c-derived B lymphoma (31) and EL4.CW (32) is an H-2d T cell lymphoma. The PHB15 DBA/2-derived matudocyte (TIB-64) was obtained from the American Type Culture Collection (Rockville, MD). BW 1100-129.237 (33) is a TCR α/β− derivative of BW517. The C3H-derived melanoma, K1735-M2 (34), was a gift of Dr. I. Fidler (M.D. Anderson Cancer Center, Houston, TX), and the F10Q clone of B16 melanoma was obtained from D. Armstrong (Emory University, Atlanta, GA). Dr. D. Accili (National Institutes of Health, Bethesda, MD) provided transformed hepatocyte cell lines generated from insulin receptor-deficient (IR−−) and IR+/− littersmates (35). B3Z, a CD8− T-cell hybridoma specific for OVA 257-264 (SIINFEKL) presented by K1, was provided by Dr. N. Shastri (University of California, Berkeley, CA). Insulin-specific T cell hybridomas (25) were prepared from C57BL/10SnJ mice immunized subcutaneously with 50 μg pork insulin emulsified in CFA. 9–12 d after immunization, lymphocytes were isolated from draining lymph nodes and cultured for 4 d at 106 cells/ml with 100–200 μg/ml pork insulin in RPMI 1640 supplemented with 0.5% fresh prepared normal mouse serum, 2 mM glutamine, 50 μM 2-ME, 10 μg/ml gentamicin, 1 mM sodium pyruvate, 50 U/ml penicillin, and 50 μg/ml streptomycin. Viable lymphocytes isolated on a Ficol gradient were fused at a ratio of 1:2 or 1:3 with T lymphoma cells and selected in RPMI 1640 supplemented with 20% FCS and 2 mM HAT. T cell hybrids were produced using BW5147 (Pb13, Pb14) or 3B2-E7 (6C5, B1A4), a CD8− transfected, TCR α/β− derivative of BW5147 (36) provided by Dr. P. Gottlieb. Hybrids were expanded in medium containing hypoxanthine and thymidine for initial screening. Primary wells containing cells secreting lymphokine after stimulation with pork insulin in the presence of C57BL/10SnJ splenocytes as APCs were subcloned by limiting dilution. Phenotypes were determined by using indirect immunofluorescence and flow cytometry. All hybridomas express TCR α/β but not TCR γ/δ. Pb13 (CD4−CD8+), 6C5 (CD4−CD8+), Pb14 (CD4+CD8−), and B1A4 (CD4+CD8+) are representative of hybridomas isolated from multiple independent fusions.

Cos Cell Transfection. Transiently transfected Cos cells expressing Qa-1a were generated using Qa-1a cDNA cloned into the mammalian expression vector pCDNA3.0 (Invitrogen Corp., Carlsbad, CA). On day 0, 3.0 × 105 Cos (37) cells were plated in 5 ml supplemented DMEM/10% FCS, in 6 cm tissue culture dishes. Cultures were aspirated on day 1 and washed twice with DMEM plus 10 mM Hepes. To each dish, 2 ml DMEM plus 10 mM Hepes was added, containing 1 μg sterile cDNA (or vector only), 500 μg DEAE-dextran, and 20 ml 10 mM chloroquine. Petri dishes were incubated for 3 h at 37°C, 7% CO2. After incubation, plates were aspirated, and 2 ml of 10% DM SO in DMEM plus 10 mM Hepes was added. Petri dishes were incubated 1–2 min, aspirated, and 5 ml DMEM/10% FBS was added. Plates were incubated for 24–48 h before cell removal by trypsinization and use in T cell assays. Qa-1a expression was measured using indirect immunofluorescence and flow cytometry with a Qa-1a-specific rabbit antiserum.
A analysis of Q α-1 Expression by Flow Cytometry. Rabbit antisera were generated with a synthetic peptide from the α2 domain of Q α-1, and the IgG fraction was purified using protein A-Sepharose affinity chromatography. Cells (10^6) were incubated with Q α-1-specific or control rabbit IgG (10–20 μg/ml) in 50 μl PBS containing 1% FCS for 30–45 min, washed, and further incubated with FITC-labeled goat anti–rabbit IgG (Southern Biotechnology Associates, Inc., Birmingham, AL). Fluorescence was analyzed using a FACScan (Becton Dickinson, San Jose, CA).

Culture Conditions and Lymphokine Assay. Cultures were performed in 96-well tissue culture plates in a final volume of 200 μl/well of RPMI 1640 supplemented with 10% FCS plus additives as described above. T-cell hybridomas (5 × 10^4 or 10^5/well) were cultured for 24 h in the presence of various antigens and APCs. In experiments with inhibitors, APCs were pretreated with inhibitor for 1 h followed by the addition of antigen followed by an additional 4 h of culture. APCs were washed and fixed by exposure to 0.5% paraformaldehyde for 10 min at 24°C, followed by three washes in serum-containing medium. Thiglycollate-induced peritoneal exudate cells were obtained through lavage of the peritoneal cavity of mice injected intraperitoneally. They were cultured for 24 h at 37°C to allow macrophages to adhere, and nonadherent cells were removed by washing before T-cell addition. In some experiments, 4.5 μm goat anti–rabbit Dynabeads (Dynal, A.S., Oslo, Norway) were added to stimulate phagocytosis (38). Lymphokine production, reflecting T-cell activation, was quantified by measuring proliferation of the IL-2–dependent T-cell line, CTL-L. Culture supernatants (100 μl) were transferred to flat-bottomed, 96-well tissue culture plates, freeze-thawed once, and cultured with 10^5 CTL-L cells/well for 24 h. Each well was pulsed with 1 μCi of [3H]thymidine during the final 18 h of culture. All experiments were performed in triplicate, and data are representative of at least two independent experiments.

Results

In multiple independent fusions, we have isolated T-cell hybridomas from pork insulin–primed nonresponder H-2^b mice that specifically recognize insulin but appear to be MHC-unrestricted based on the observation that they can be stimulated in the presence of MHC-mismatched APCs. These hybridomas express α/β TCRs, and CD8 is expressed only if a CD8^+ fusion partner is used to generate the cell lines. They do not express CD4 or γ/δ TCRs (data not shown). The T cells might recognize insulin peptides presented by nonpolymorphic MHC proteins, peptides bound to an alternative site in MHC class II molecules, or they may be stimulated through a mechanism completely independent of MHC molecules. The possibility that insulin may directly stimulate the T cells through hormonal receptors or other means was excluded because insulin does not activate the T cells in the absence of accessory cells (see below). We reported previously that insulin B-chain peptides can bind to an alternative site overlapping the SEB-binding site outside of the peptide-binding groove in multiple different class II proteins (39). Therefore, the potential requirement for MHC class II molecules was addressed by evaluating the capacity of spleen cells from class II-deficient mice to present insulin to representative T-cell hybridomas. As expected, these cells are unable to present insulin to control, IA<sup>-</sup>-restricted T cells (Fig. 1 A). In contrast, the antigen presentation activity of class II–negative spleen cells was equal to that of wild-type B6 cells in experiments with MHC-unrestricted T cells (Fig. 1 B). The potential role of MHC class I molecules was investigated using spleen cells from β2m knockout mice, which were observed to present insulin to the IA<sup>-</sup>-restricted but not the unrestricted T cells (Fig. 1, C and D). These results suggested that the unrestricted T cells recognize insulin peptides presented by a nonpolymorphic β2m-dependent class I or class I–like molecule.

The family of β2m-associated class I-related molecules includes the classical class Ia proteins K, D, and L, class Ib proteins encoded in the Q, T, and M regions of the MHC, and molecules such as CD1 that are not encoded in the MHC. Experiments with APCs from a panel of inbred mice demonstrated a correlation between the identity of alleles encoded in the T region with the capacity to present insulin to Pb13 and 6C5 T-cell hybridomas (Fig. 2). Antigen presentation did not correlate with class Ia loci or the Q or M regions. The restriction element was definitively mapped using congenic strains differing only in the T region. APCs from A/J-Tia^b and B6-Tia<sup>b</sup> (T<sup>b</sup>) mice presented insulin to 6C5 T cells, whereas those from congenic T<sup>a</sup> strains did not (Fig. 2 B). B6-Tia<sup>b</sup> spleen cells were fully capable of presenting insulin to control IA<sup>-</sup>-restricted B1A4 T cells, ruling out any intrinsic defect in the general capacity to present insulin to T cells.

Figure 1. MHC class I- and class II–dependent recognition of insulin. T cell hybridomas (A) Pb14, (B) Pb13, (C) B1A4, and (D) 6C5 (10^5/well) were cultured with 10^5 spleen cells in the presence (black bars) or absence (white bars) of 30 μM pork insulin. Splenic APCs were obtained from β2m-deficient, MHC class II–deficient, or wild-type B6 mice. Results represent lymphokine production measured as described in Materials and Methods. Pb14 and B1A4 are representative of MHC class II–dependent T cells, whereas Pb13 and 6C5 are representative of β2m-dependent T cells isolated from pork insulin–primed B10 mice.
The T region includes at least five genes with open reading frames that are transcribed or encode cell surface proteins (2). TCR γ/δ T cell lines can recognize the T22 gene product (40, 41). Products of the related T3/T18 genes can be recognized by TCRαβ cytotoxic T cells and act as transplantation antigens when expressed as transgenes under the control of the H-2Kb promoter (42, 43). The T23 gene (formerly known as gene 37) encodes Qa-1, which can be recognized by alloreactive TCRαβ CTLs (10–13, 17, 18). Recent work also demonstrates that Qa-1β can present antigen(s) from the intracellular pathogen L. monocytogenes to αβ CTLs (24). We used transfected cell lines to investigate the possibility that Qa-1β was the restriction element recognized by our β2m-dependent, insulin-specific T cells. The Qa-1β-expressing L cell transfectant, L-g37, but not control transfectants, presented insulin to 6C5 T cells (Fig. 3A). L-g37 did not present insulin to control, IAb-restricted B1A4 T cells. The role of Qa-1β was confirmed using transient Cos cell transfectants (Fig. 3B), which ruled out the possibility that we had inadvertently selected cells expressing Qa-1–unrelated molecules required for stimulation of 6C5 T cells during isolation of the cloned, stably transfected L-g37 cell line. We conclude that the unrestricted T cell hybridomas isolated from pork insulin–primed H-2b mice actually recognize insulin presented by the oligomorphic class Ib protein, Qa-1β.

The determinant specificity of pork insulin–induced IAb- and Qa-1β–restricted T cells was investigated (Fig. 4, and data not shown). Insulin contains a 30 amino acid B chain peptide that is linked through two disulfide bonds to a 21 amino acid A chain. The IAb-restricted T cells were found to recognize a determinant within residues 17–30 of the B chain. The Qa-1β–restricted T cells fully cross-react with beef insulin, which differs from pork insulin by two residues in the A chain loop that is formed by a disulfide bond between cysteines A6 and A11. The conclusion that the COOH terminus of the B chain is a critical component of the determinant was suggested by the observation that the T cells do not respond to desoctapeptide insulin, a tryptic peptide missing the COOH-terminal eight amino acids of the B chain. The antigenicity of sulfonated B chain and a synthetic B(1-30) peptide demonstrated that the determinant is fully localized in the B chain. The chemical status of the cysteine side chains does not appear to be important, because performic acid–oxidized insulin is antigenic. The B1α amino group and the B29 ε amino group are not essential, since biotin-N-hydroxysuccinimide derivatives re-
tain activity. By contrast, IAβ-restricted B1A4 cells do not respond to biotin-insulin, suggesting that the B29 Lys is a critical component of this determinant.

The only previously characterized peptide determinant recognized by Qα-1-restricted T cells is derived from the leader sequence of MHC class Ia D or L molecules (18). The Qdm nonamer (AM APR TLLL) optimally stimulates a large fraction of Qα-1β-restricted allogeneic CTLs. Peptide binding experiments with substituted Qdm analogues suggest that P2 and P9 may be dominant anchor positions (44). These findings support the assumption that the peptide specificity of Qα-1 is similar to class Ia proteins, with preference for nonamers bearing appropriate residues at key anchor positions. Given this assumption, we were surprised to find that none of a set of overlapping nonameric peptides from the COOH terminus of the pork insulin B chain stimulated Qα-1β-restricted T cells (Fig. 4). We considered the possibility that the T cells preferentially recognize longer peptides. Aldrich et al. (18) reported that 10- and 11-mer Qdm derivatives extended at the COOH terminus stimulate allogeneic CTLs almost as well as the nonamer. However, none of a set of NH2-terminally truncated B chain peptides beginning with B(6-30) stimulate our Qα-1β-restricted T cells (Fig. 4). Thus, it appears that the determinant recognized by these cells includes much if not all of the full 30 amino acid B chain.

Given the near ubiquitous expression of the insulin hormonal receptor, it was important to rule out the possibility that the T cells recognize a determinant unrelated to insulin that is induced through the hormonal activity of insulin. This possibility was unlikely, because isolated insulin B chain has no hormonal activity (45) while retaining a catalytic activity. By contrast, IAβ-restricted B1A4 cells do not respond to biotin-insulin, suggesting that the B29 Lys is a critical component of this determinant.

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somal compartments (58). Given evidence for a TAP-independent, chloroquine-sensitive endosomal processing pathway in presentation of insulin by Qa-1b, we evaluated the antigen presentation activity of spleen cells from invariant chain-deficient mice (Fig. 7A). Presentation of myoglobin to control IAα-restricted HMb4.2 T cells was abrogated in the absence of invariant chain. In contrast, invariant chain expression had no effect on presentation to 6C5 T cells. H-2M catalyzes peptide-exchange reactions in MHC class II molecules localized in endosomal compartments (26, 59, 60). The possibility that H-2M can catalyze peptide exchange in class I molecules has not been addressed. We observed no significant difference in the antigen-presenting activity of H-2M-deficient and control spleen cells in experiments with 6C5 T cells (Fig. 7B). In contrast, control but not H-2M-negative APCs presented myoglobin to HM b4.2 T cells. In preliminary in vitro experiments, we found no evidence that the human homologue of H-2M, HLA-DM, can catalyze peptide-exchange reactions in purified MHC class Ia proteins (data not shown). We conclude that invariant chain and H-2M are not required for presentation of insulin to Qa-1b-restricted T cells.

The cytosolic and noncytosolic exogenous class I antigen processing pathways are selectively localized in macrophages (46–48) and dendritic cells (49–51). MHC class II-negative cells, T cells, and B cells in general are unable to present exogenous protein determinants to class I-restricted T cells (47, 52). Exceptions have been reported, but they appear to be associated with specialized forms of antigen or specialized mechanisms of antigen uptake (61–63). We found that a variety of nonphagocytic cell lines present insulin to Qa-1b-restricted T cells, with an efficiency similar to macrophages or spleen cells (Fig. 8). Antigen presentation activity correlated with Qa-1b expression as measured by flow cytometry. 6C5 T cells do not express cell-surface Qa-1b.
Qa-1b (data not shown), explaining their inability to respond to insulin in the absence of APCs.

The previously characterized exogenous class I antigen processing pathways are strongly stimulated by phagocytosis (38, 57, 64). H-2Kb-restricted B3Z T cells respond weakly to OVA presented by peritoneal macrophages (Fig. 9). This response is markedly enhanced in the presence of 4.5-μm particles as described previously (38). By contrast, macrophages efficiently present insulin to Qa-1b-restricted T cells in the absence of phagocytic stimuli, and the response is only slightly enhanced in the presence of 4.5-μm particles (Fig. 9). Thus, it appears that Qa-1b has access to a novel antigen processing pathway that is constitutively active in a wide range of cell types and is not stimulated by phagocytosis.

Discussion

We describe the isolation and initial characterization of TCR α/β T cell hybridomas from H-2b mice that recognize pork insulin presented by the nonclassical class I protein, Qa-1b. The restriction element was localized in experiments with T region congenic mice and identified using transfected cell lines expressing Qa-1b. Blocking experiments with T region congenic mice and identified using transfected cell lines expressing Qa-1b. This response is only slightly enhanced in the presence of 4.5-μm particles (Fig. 9). Thus, it appears that Qa-1b has access to a novel antigen processing pathway that is constitutively active in a wide range of cell types and is not stimulated by phagocytosis.

The dominant peptide, Qdm, recognized by Qa-1b-restricted T cells is derived from the leader sequence of D or L MHC class Ia proteins (18). The natural form of this peptide isolated by acid extraction of Con A-activated lymphoblasts is a nonamer, and it is the major peptide associated with Qa-1b in these cells (18, 19). Similar results were obtained from analysis of peptides eluted from purified chimeric class I molecules expressing the α1 and α2 domains of Qa-1b and the α3 domain of D. The limited heterogeneity of Qa-1b-associated peptides may reflect a relative shortage of peptides in the ER with the appropriate sequence motif, or it could result from unknown constraints on the mechanism of peptide loading by Qa-1 molecules. However, the dominance of Qdm is consistent with the idea that Qa-1b preferentially binds nonameric peptides similar to MHC class Ia proteins. Therefore, it is surprising that we were unable to identify short peptides from the insulin B chain that could stimulate our Qa-1b-restricted T cells. It is clear that the COOH terminus of the B chain is essential for T cell activation. Desoctapeptide insulin and B(1-22), missing the eight COOH-terminal residues of the B chain, have no activity. However, the NH2-terminus of the B chain also appears to have residues required for T cell activation. B(6-30) and shorter NH2-terminally truncated B chain peptides do not stimulate the Qa-1b-restricted T cells. It remains possible that the B chain is further processed in APCs, and that a covalently modified peptide represents the final species that associates with Qa-1b.

The possibility that Qa-1b may be especially suited to bind and present peptides of unusual length cannot be excluded. Peptide binding assays with Qa-1b-expressing cells have been used to identify anchor residues in the Qdm sequence (20, 44); however, the capacity of longer peptides to bind Qa-1b has not been directly examined. Aldrich et al. (18) reported that 8-, 10-, and 11-mer variants differing from Qdm at the COOH-terminus stimulate alloreactive CTLs with only slightly reduced potency, whereas 7- and 12-mer peptides are 50-100-fold less potent but still demonstrate substantial activity in cytotoxicity assays. Longer peptides were not tested. Imani and Soloski (22) demonstrated that a tryptic digest of M. bovis 65-kD heat shock protein, containing peptides of various lengths, stabilizes cell surface Qa-1b molecules. The determinants recognized by recently described Qa-1b-restricted, L. monocytogenes-specific CTLs has yet to be defined (24). Qa-1b (and Qa-1a) differ from other class I molecules by serines at positions 143 and 147 instead of threonine and tryptophan, respectively (15, 16, 65). These positions represent two of four highly conserved residues in the F pocket that form hydrogen bonds with the COOH terminus of class I-bound peptides, limiting their length and contributing substantially to the stability of the complexes (66). The consequence of these substitutions on the binding specificity and stability of Qa-1b-peptide complexes is unknown, but it is possible that interactions involving the COOH terminus of bound peptide play a smaller role in Qa-1 than other class I molecules. Mouse CD1 preferentially binds peptides 14–24 amino acids in length to form complexes that can be recognized specifically by TCR α/β CTLs, providing an example of a class I-related molecule that binds long peptides.
reactions in post-ER compartments after relatively short
rate in cells (15, 22), and thus there may be a substantial re-
potent for export to the cell surface or endosomal compart-
usually long peptide antigens.

The results of experiments with inhibitors and APCs from TAP knockout mice suggest that a noncytosolic pro-
cessing pathway is used to present insulin to Qa-1b–restricted
from TAP knockout mice suggest that a noncytosolic pro-
compartments, where it can participate in peptide-exchange
reactions. The class I–related human CD1b protein is selec-
tively targeted to endosomes through a tyrosine-based target-
ing motif in the cytoplasmic domain (6). A similar motif is
present in the cytoplasmic domain of Qa-1b, and experiments
are in progress to evaluate the requirement for the cyto-
plasmic domain in stimulating insulin-specific T cells. Immu-

formaldehyde and paraformaldehyde fix the intracellular
compartments in TAP-deficient cells. This interpretation would
participate in peptide-exchange reactions. As
noted above, Qa-1b has a very rapid turnover
rate in cells (15, 22), and thus there may be a substantial re-
duction in the quantity of molecules available for exchange
reactions in post-ER compartments after relatively short
incubations with BFA.

Both cytosolic and noncytosolic pathways have been de-
scribed for the presentation of exogenous protein antigens
to class I–restricted T cells (52, 53). In general, these path-
ways are selectively active in macrophages (46–48) and a
subpopulation of dendritic cells (49–51) but not B cells, T
cells, or other class II–negative cell types. Therefore, it is
striking that a wide variety of MHC class II–positive and
negative cell lines derived from different tissues are able to
present insulin to Qa-1b–restricted T cells with efficiencies
similar to peritoneal macrophages or unfractionated spleen
cells. Presentation of exogenous proteins by class I mole-
cules in macrophages is markedly enhanced under condi-
tions favoring uptake of antigen by phagocytosis (54, 56,
57, 64) or macrophagocytosis (48). Rie's et al. and Ger-
main (38) demonstrated that antigen does not need to be
physically coupled to particles to stimulate presentation of
OVA to Kb-restricted T cells. This suggests that phagocyt-

In addition, antigen-pulsed APCs stimulate substantially lower
T cell responses compared with those observed in the con-
tinuous presence of antigen. TCRa/b of T cell hybridoma that recognizes
synthetic copolymer poly(Glu50Tyr50) presented by Qa-1b
on the surface of fixed APCs. This polymer has been shown to
stabilize cell surface Qa-1b molecules. Thus, several observations support the
possibility that Qa-1b may be able to bind and present usu-
ally long peptide antigens.

The results of experiments with inhibitors and APCs from TAP knockout mice suggest that a noncytosolic pro-
cessing pathway is used to present insulin to Qa-1b–restricted
T cells. Chloroquine completely inhibited antigen presents-
tion, implicating endosomal compartments in the pro-
cessing pathway. APCs from TAP-deficient animals were
almost as efficient as cells from control mice in antigen pre-
sentation. The slightly reduced activity of TAP-deficient APCs can be explained by a reduction in the quantity of
Qa-1b–peptide complexes assembled in the ER and com-
petent for export to the cell surface or endosomal compart-
ments. Formation of Qa-1b–Qdm complexes is TAP-
dependent (69). Since this is likely to be the major peptide
complex assembled in the ER (19, 70), one would expect a
reduction in Qa-1 transport in TAP-negative APCs. Bio-
chemical evidence has also been obtained demonstrating
reduced maturation and transport of Qa-1b molecules in
activated T cells from TAP-negative mice (13). However,
a substantial fraction of alloreactive CTLs specific for Qa-1b
recognize TAP-independent determinants (69), and Qa-1b
transport is only partially inhibited in TAP-deficient spleen
cells (13). Thus, a cohort of Qa-1b molecules should still be
available for peptide-exchange reactions in post-ER com-
partment in TAP-deficient cells. This interpretation would
also account for the observation that antigen presentation is
substantially inhibited by BFA, which blocks transport to
post-ER compartments. Qa-1b has a very rapid turnover
rate in cells (15, 22), and thus there may be a substantial re-
duction in the quantity of molecules available for exchange
reactions in post-ER compartments after relatively short
incubations with BFA.

(67). In addition, the three-dimensional structure of a pep-
tide extending out of the COOH-terminal end of the pep-
tide-binding site of a class Ia protein has been reported
(68). The conserved residues that form hydrogen bonds with the peptide NH2 terminus in class I molecules are
conserved in Qa-1 (15, 16, 65). Thus, if the insulin B chain
extends out of the NH2-terminal end of the peptide-bind-
ing site in Qa-1, the complex is likely to be very unstable
with a rapid rate of dissociation. This possibility must be
considered because insulin derivatives with a chemically mod-
ified B chain α amino group retain activity in T cell assays.
In addition, antigen-pulsed APCs stimulate substantially lower
T cell responses compared with those observed in the con-
tinuous presence of antigen. TCRgδ T cell hybridoma that recognizes
synthetic copolymer poly(Glu50Tyr50) presented by Qa-1b
on the surface of fixed APCs. This polymer has been shown to
stabilize cell surface Qa-1b molecules. Thus, several observations support the
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incubations with BFA.
it is likely that these complexes are substantially less stable at 37°C. Given the assumption that Qa-1α–Qdm complexes represent the most stable Qa-1α–peptide complexes, it is likely that peptide-receptive Qa-1β molecules are generated rapidly under physiological conditions.

The function of insulin-specific, Qa-1β–restricted T cells remains to be determined. H-2b mice are nonresponders to pork insulin, generating little if any antibody after immunization with this antigen (71). Beef insulin, differing from pork insulin, generating little if any antibody after immunization (73). In addition, Jiang et al. (23) have described TCR α/β CTLs responsible for the peripheral deletion of CD8+ T cells that is observed after injection of SEB. These CTLs apparently recognize TCR Vβ determinants presented by Qa-1α (23). Thus, Qa-1α could have a special role in mediating regulatory interactions between T cells.

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