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Evidence for antigen presentation by the class Ib molecule, Qa-1

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There are several lines of evidence that support the notion that the class Ib molecule called Qa-1, determined by the *T23* gene, can present antigen to the T-cell receptor. These lines of evidence consist of both *in vitro* and *in vivo* results. In this short review, we will review these data and hypothesize on the function of Qa-1.

The Qa-1 antigen was first identified when an antiserum against the previously described class Ib molecule, TL (thymus leukaemia antigen), was found to react with peripheral T cells (Stanton and Boyse, 1976). The target could not be TL, because TL expression was limited to thymocytes and leukaemia cells. The gene coding for the Qa-1 antigen was mapped to the T region and subsequently identified as the previously isolated gene 37 (Lalanne *et al.*, 1985; Wolf and Cook, 1990). More recently, the name of this gene was changed to *T23* to conform with current MHC nomenclature and indicate its approximate position in the *T* region.

Genetic and molecular characteristics of Qa-1

The sequence of *T23* has been determined (Transy *et al.*, 1987). The nucleotide sequence shows about 72% homology with the class Ia molecule, H-2K. The domain organization is very similar, with conservation of its glycosylation sites, position of its cysteine residues, and its interaction with β_2 microglobulin (Prochnicka-Chalufour *et al.*, 1989). Residues known to be important in the interaction with CD8 have also been found (Salter *et al.*, 1990). These similarities indicate that Qa-1 has all of the structural requirements necessary for antigen presentation including a functional cleft for binding with peptide.

As is the case for most class Ib molecules, Qa-1 is relatively non-polymorphic. Two alleles have been defined serologically, *Qa-1^a and Qa-1^b* (Flaherty *et al.*, 1981; Stanton *et al.*, 1981).

Two additional alleles, *Qa-1^c* and *Qa-1^{1d}*, have been identified by CTL responses, although the genes encoding these alleles have not yet been cloned (Aldrich *et al.*, 1986). Additional alleles have been identified in wild mice (Fischer-Lindahl, 1986). All strains, even wild ones, possess a serologically detectable Qa-1 molecule (Nakayama *et al.*, 1990). This is in contrast to other *Q* and *T* region genes that are often deleted.

Qa-1 is expressed in a broad range of tissues, including liver, kidney, spleen, thymus, placenta, brain and heart, as demonstrated by RNase protection assays (Transy *et al.*, 1987). In addition, mRNA could be detected in a number of cell lines derived from various origins including fibroblasts, hepatomas, thymomas and myelomas. This tissue distribution is more similar to that of the class Ia molecules than to the limited tissue expression of some other Q and T region genes. Thus, Qa-1 has more similarities to functional antigen-presenting molecules, such as H-2K, H-2D and H-2M3, than it does to presumed non-functional ones.

Allogeneic response to Qa-1

The first indication that Qa-1 possessed antigen-presentation function was the observation that an allogeneic response to Qa-1 was unrestricted by either H-2K or H-2D (Fisher Lindahl, 1979; Kastner and Rich, 1979; Klein and Chiang, 1978). This observation meant that the whole Qa-1 molecule (rather than a peptide derived from it) was involved in recognition by the T-cell receptor. In these reports, the allogeneic response against non-class Ia molecules is much weaker than for H-2K or H-2D and requires *in vivo* priming and subsequent *in vitro*

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challenge with the alloantigen. Recognition of alloantigens appears to be peptide-dependent, as evidenced by the failure of mutant cell lines, defective in antigen processing and transport, to serve as allogeneic targets (Heath *et al.*, 1991). Therefore, although the nature of the peptide(s) is unknown, it is presumed that Qa-1 presents peptide to alloreactive T cells.

Recognition of Qa-1 by γδ T cells

In 1989, Vidovic *et al* (1989) were the first to observe a clear-cut role of antigen presentation for Qa-1. They showed that a T helper cell hybridoma clone (DGT3) recognized a copolymer of poly GT. Activation of this clone was assessed by measuring IL2 production. The response of these cells was specific for the GT copolymer because no activation was seen in the presence of two other related antigens, poly GAT or poly GA. Activation was inhibited only by anti-Qa-1 antibodies but not by antibodies to class Ia molecules (K, D, or L) or MHC class II molecules. The inhibition was allele-specific because antibody to Qa-1^b was inhibitory while antibody to Qa-1^a was not. Antibody against another class Ib molecule, Qa-2, also failed to inhibit activation. The DGT3 clone was found to express γδ T-cell receptors and have a $CD4^-CD8^-$ phenotype.

Soon after the observations of Vidovic *et al* (1989), Imani and Soloski (1991) investigated the effects of heat shock on Qa-1 expression. Heat shock of *T23*-transfected cells at 42°C for 1 h greatly increased the surface expression of Qa-1. However, there was no increase in the surface expression of the class Ia molecule, H-2K. These results suggested that stabilization of Qa-1 might occur through the binding of peptides generated during a heat-shock response. To further evaluate this idea, the *T23*-transfected cells were first incubated at room temperature for 24 h to stabilize empty class I molecules, and then incubated with a tryptic digest of *Mycobacterium bovis* hsp65 or BSA as a control. When the cells were returned to 37°C, hsp65 stabilized cell surface expression of Qa-1 while BSA did not. Incubation with the synthetic copolymer poly GT also resulted in an increase of Qa-1 on the cell surface. Thus, Imani and Soloski (1991) hypothesized a role for Qa-1 in the presentation of heat shock proteins to the immune system as a means of identifying cells that had been altered by stress or infection.

Qa-1 presentation of a signal peptide

Even though all strains express Qa-1, some strains such as $H\text{-}2^k$-bearing ones, express it in lower amounts (Fischer-Lindahl, 1983; Flaherty *et al.*, 1981). The gene or genes determining this lower expression was mapped to the *H-2D* region (Flaherty et al., 1981). These observations were followed by an elegant series of experiments by Aldrich et al (1988) that elucidated the nature of this phenomenon. Allogeneic Qa-1-specific CTLs could be divided into those that were influenced by the *H-2D* region and those that were not. They were denoted Qdm^+ and Qdm^- standing for Qa-1 determinant modifier. Lymphoblasts from C3H($H\text{-}2^k$) mice expressing a D^d or L^d transgene could serve as targets for Qdm-dependent CTLs, suggesting that expression of either of these genes conferred a Qdm^+ phenotype. Transfection of the L^d gene into human lymphoblastoid cells also resulted in expression of the Qdm^+ phenotype. The relevant peptide was further characterized by transfecting smaller pieces of L^d into these cells. Part of the leader sequence of L^d having the amino acid sequence, AMAPRTLLL, resulted in conversion from a Qdm^- to Qdm^+ phenotype (Aldrich *et al.*, 1994). Mice of the $H\text{-}2^k$ haplotype do not express an *L* gene and the D^k gene signal peptide differs from this L^d sequence by a valine instead of an alanine in the third position. Thus it is hypothesized that this single amino acid change is sufficient to cause inefficient presentation of this peptide. Interestingly, this same peptide is part of the leader sequence of the E3 glycoprotein of murine hepatitis virus (Luytjes *et al.*, 1988). Although it has yet to be demonstrated that Qa-1 is involved in the immune response to this virus, the possibility that Qa-1 functions in the presentation of relevant peptides during infection certainly seems plausible. However, no peptide motif has yet been described for Qa-1 so the possible scope of peptides presented by Qa-1 has not yet been determined.

Tap-dependent and independent recognition of Qa-1

Aldrich *et al* (1992) found that recognition of the L^d signal peptide by Qdm-dependent CTLs required a functional Tap transporter. They observed that Qdm-dependent CTLs were unable to lyse RMA-S targets which are defective in this transporter. Transfection of this mutant cell line with a functional *Tap-2* gene restored the recognition of Qa-1 by these CTLs. This group of investigators also described some of the properties of Qdm-independent CTLs. These CTLs could be divided into two classes — those that were Tap-dependent and those that were Tap-independent as evidenced by their ability to lyse RMA vs RMA-S cells. The presence of a Tap-independent pathway is in sharp contrast to the almost exclusive Tap-dependent pathway utilized by class Ia molecules. Other class I-like molecules have been shown to utilize a Tap-independent pathway. For example, CD1 has

been shown to present *M. tuberculosis* antigens to a $CD4^-CD8^-$ T cell line in a Tap-independent manner (Porcelli *et al.*, 1992). As an alternative to the idea of a Tap-independent pathway for Qa-1-presented peptides, it could be argued that Tap-independent CTLs recognize empty Qa-1 molecules rather than peptide-loaded ones. This possibility seems unlikely based on the observation that stabilization of empty Qa-1 molecules by overnight incubation at room temperature did not increase the reactivity of Tap-independent CTLs (Aldrich *et al.*, 1992).

Role for Qa-1 in downregulation of immune responses

One of the most recent and intriguing pieces of information about Qa-1 concerns its possible function as a regulator of immune responses. Studies by Jiang *et al.* (1995) suggest that Qa-1 plays a role in tolerance induction through deletion of specific T-cell subsets. Administration of the superantigen staphlococcus enterotoxin B (SEB) to mice results in the proliferation and subsequent deletion of $CD4^+$ and $CD8^+$ T cells that express TCR Vβ8 chains (Kawabe and Ochi, 1991). The $CD4^+V\beta8^+$ T cells are deleted 30-40% below baseline levels and remain there for 3 weeks, while the $CD8^+V\beta8^+$ T cells simply return to baseline levels but are not further deleted. Jiang *et al* (1995) demonstrated that this depletion of $CD4^+V\beta8^+$ T cells did not occur in mice lacking $CD8^+$ T cells either brought about by depletion with anti-CD8 antibody or in β_2-microglobulin knockout mice lacking $CD8^+$ T cells (Zijlstra, 1990). Thus they hypothesized that $CD8^+$ cells were directly responsible for the decline in $CD4^+V\beta8^+$ T cells.

They further demonstrated that $CD8^+$ T cells from SEB-primed mice could be restimulated *in vitro* to specifically kill autologous $CD4^+$ T cells carrying Vβ8 TCR chains. CTL reactivity was specific for $CD4^+V\beta8^+$ cells because $CD4^+V\beta8^-$ targets were not lysed. In addition, Vβ8-specific killing could be inhibited by anti-Qa-1 antiserum, but not by antibodies against classical class I molecules (K, D or L). The data suggest that Qa-1 on $CD4^+$ T cells presents Vβ8 or some peptide derived from this molecule to autologous $CD8^+$CTLs, resulting in deletion of this cell population. Other superantigens result in deletion of $CD4^+$ cells expressing different Vβ chains. Whether Qa-1 or other class Ib molecules are involved in these other responses has yet to be investigated.

Conclusions and speculations

With the exception of the H-2M3 molecule, the functions proposed for the non-classical class I MHC antigens are speculative. Theories have been derived based on various *in vitro* assessments of function in contrived circumstances such as in the use of transfected cells or incubation with synthetic peptides or digests of heat shock proteins. The true *in vivo* function of class Ib molecules will be much more difficult to address. There are two primary schools of thought regarding the function of the class Ib molecules. One side believes that these molecules have an active role in the immune response, either in a manner similar to the class Ia molecules or possibly in special situations or tissues. For example, Pamer *et al* (1992) and Kurlander *et al* (1992) demonstrated that one of the M region class Ib molecules, H-2M3, presents a *Listeria monocytogenes* peptide to T cells. Non-immunologic functions have also been postulated for some of the class Ib molecules such as the work of Tian *et al* (1992), suggesting the Q-region-encoded antigen, Qa-2, is involved in embryonic development. Another non-immunologic function has been proposed by Rothenberg and Voland (1996). This group suggests a role for the non-classical MHC antigens in iron absorption.

The other possibility put forth by Klein and Figueroa (1986) is that these molecules have no function at all and are simply part of the "evolutionary junkyard" of DNA. Old and abandoned class I genes are found mutated and non-functional. As a corollary to this hypothesis, some have proposed that this region may also be the breeding ground for new class I molecules possibly through donation of pieces of sequences into other functional class Ia molecules (Flavell *et al.*, 1986). The answer probably lies somewhere between these two alternatives — some class Ib molecules having functional roles and others not.

In the case of Qa-1, there is increasing evidence to suggest that the Qa-1 molecule plays an active role in the immune response. Structural studies of Qa-1 indicate that it is structurally homologous to the class Ia molecules which are known to function in antigen presentation to $CD8^+$ T cells. Domain organization of Qa-1 indicates that it retains a groove that could potentially bind peptide. It also contains residues known to be important in the interaction with the CD8 coreceptor on CTLs. These structural similarities provide the first piece of evidence that Qa-1 functions in antigen presentation.

A large proportion of the allogeneic CTLs generated against Qa-1 require the processing of the L^d signal peptide or its like. These CTLs recognize Qa-1 in association with processed self MHC-derived peptides. The relevance of this observation is uncertain, but it lends further support to the ability of Qa-1 to bind and present peptides to T cells.

The demonstration that γδ T cells can be generated that recognize Qa-1 in association with a syn-

thetic foreign antigen provides some support for the idea that class Ib molecules can serve as restriction elements for γδ T cells. Moreover, the possibility that Qa-1 presents peptides resulting from cell stress is supported by the observation that heat shock or incubation with hsp65 tryptic digests increases the cell surface expression of Qa-1. The responsive cells may be γδ T cells which have previously been shown to be activated by heat shock peptides.

The most recent evidence that Qa-1 can function in antigen presentation is the regulation of cell populations in superantigen-induced models. Qa-1 appears to play a role in the elimination of $CD4^+V\beta8^+$ cells after SEB administration by presenting peptide to autologous Vβ8-specific $CD8^+$ T cells. This apparent downregulation of responses mediated by Qa-1 suggests that Qa-1 could have a function in the modulation of anti-self responses. For example, Qa-1 and possibly other class Ib molecules could function to present peptides derived from MHC, the T-cell receptor and other molecules associated with an active immune response. Cytokines and heat shock proteins may also fall into this category. Thus, when an immune response becomes overwhelming and counterproductive, active $CD8^+$ T cells, targeted for these immune peptide containing *Q* and *T* region molecules, are then set into action. This hypothesis would explain the observations of Aldrich *et al* (1988) on Qdm as well as the more recent work of Jiang et *al* (1995).

Based on the various studies reviewed here, there is strong albeit circumstantial evidence that Qa-1 has a functional role in antigen presentation. A role for Qa-1 in allogeneic responses, presentation of heat shock proteins and self peptides as well as in immune regulation have all been demonstrated. How these findings relate to the *in vivo* function of Qa-1 has yet to be resolved.

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Multiple products of class Ib Qa-2 genes which ones are functional?

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Introduction

One of the hallmarks of the vertebrate major histocompatibility complex (MHC) class I gene families is their remarkable plasticity. The number of class I genes, their organization, structural characteristics and their patterns and levels of expression differ from species to species. It is frequently impossible to assign orthologous relationships between class I genes isolated from different organisms. Hence, it has been proposed that the molecular evolution of the class I MHC locus occurs at a higher rate than evolution of genes at other chromosomal regions (Kasahara *et al.*, 1995, Flajnik, this Forum). This is particularly well documented in a murine system, where the class Ib gene families are continuously undergoing expansions, contractions and rearrangements due to gene duplications, deletions and various recombination events. For example, drastic differences in the genetic content of the class Ib clusters have been identified between closely related substrains of BALB/c mice that diverged from each other as recently as ≈70 years ago (Flaherty *et al.*, 1985, Mellor *et al.*, 1985, Nakayama *et*

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