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Review

The expanding role of murine class Ib MHC in the development and activation of Natural Killer cells

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ABSTRACT

Major Histocompatibility Complex-I (MHC-I) molecules can be divided into class Ia and class Ib, with three distinct class Ib families found in the mouse. These families are designated as Q, T and M and are largely unexplored in terms of their immunological function. Among the class Ib MHC, H2-T23 (Qa-1b) has been a significant target for Natural Killer (NK) cell research, owing to its homology with the human class Ib human leukocyte antigen (HLA)-E. However, recent data has indicated that members of the Q and M family of class Ib MHC also play a critical role in the development and regulation NK cells. Here we discuss the recent advances in the control of NK cells by murine class Ib MHC as a means to stimulate further exploration of these molecules.

1. Introduction

There are two types of response that occur following immune stimulation and these have been termed innate and adaptive. As the name implies the innate response occurs rapidly, provides local control of infection and assists the generation of a robust adaptive response. This is a critical component of immunity as it prevents rapid dissemination of pathogens and provides the adaptive immune response with the time it needs to generate the appropriate response.

NK cells play an important role in the innate immune system, which is highlighted in numerous publications (Andrews et al., 2010; Gianchecchi et al., 2018; Zhang and Huang, 2017). Despite the central role of these cells in immunity the full extent of how NK cells can respond to stimuli is not fully understood. What is clear is that NK cells receive their instruction from molecules encoded by the major histocompatibility complex (MHC). Class I MHC (MHC-I) proteins interact with specialised receptors on the surface of the NK cell, which then decode this signal prior to unleashing their immune potential. In this regard, class I MHC is a focal point for NK cells as it dictates whether or not these cells will become active.

The receptors that recognise class I MHC and the signals they transmit are discussed in detail below. Prior to a discourse on these interactions it is pertinent to explore the biology of NK cells and generalised mechanisms that control self reactivity and activation of these cells. During homeostasis NK cells remain in a state of “restrained activation”, a condition that is central to the rapid response of these cells following challenge. Upon “full” activation, NK cells induce direct cytotoxicity of target cells via perforin and granzymes (Voskoboinik et al., 2015) and produce pro-inflammatory cytokines, of which interferon gamma (IFN-γ) is the main effector (French and Yokoyama, 2003). This not only results in the death of the target cell, but also stimulation and amplification of the surrounding immune system. These attributes mean that NK cells are critically involved in the response to microbial infections (Andrews et al., 2003; Andrews et al., 2001; Andoniou et al., 2005) and tumour surveillance (Morvan and Lanier, 2016). The balance between “restrained” and “full” activation is regulated by the presence of ligands on the target that are bound by inhibitory and activating NK cell receptors. During homeostasis, NK cells receive a predominantly inhibitory signal. However, during periods of stress, such as infection or neoplastic transformation, the inhibitory signals are reduced, shifting the NK cell from “restrained” to “fully” activated and unleashing the suite of NK cell effector functions.

As a generalisation, this description of NK cell activity suggests an “on” or “off” functionality, however we now understand that this is not true and the control of NK cell function involves a high level of precision. Indeed, in more recent years several studies have suggested that “licensing” plays a critical role in the development of NK cells (Kadri et al., 2015). Much like the name implies, this model suggests that for an NK cell to become fully activated it must first be able to respond to an inhibitory signal, received through the interaction of an NK cell receptor with members of the class I MHC. While this seems counter-intuitive, it ensures that the NK cell is able to respond to inhibitory signals, and therefore will only become activated when appropriate.

As it is a critical instructor of NK cell responses, study of the class I

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MHC has yielded important information regarding NK cell biology, however a large family of class I MHC remain unexplored. This review will cover what is known about class I MHC across mice and humans and how they regulate NK cell function and activation.

2. The major histocompatibility complex

2.1. Class I MHC

In humans, MHC molecules are termed Human Leukocyte Antigen (HLA) and, designating the H istocompatibility-2locus, are termed H-2 in mice (Snell, 1948). Class I MHC molecules are further subdivided into classical (class Ia) and non-classical (class Ib) groups. In humans the class Ia molecules are designated as HLA–A, HLA-B and HLA-C and in the mouse these same molecules are termed H-2K, H-2D and H-2L. The class Ia MHC molecules are highly polymorphic glycoproteins that are expressed in most nucleated cells and they are largely recognised for their ability to present antigenic peptides to cytotoxic or CD8 T cells. This occurs via the specialized structure of the MHC, which consists of an α-chain and a non-covalently associated beta-2-microglobulin polypeptide (β2m). The α-chain has 3 extracellular domains termed α1, α2 and α3 with a groove formed between the α1-α2 domains that binds peptides (Fig. 1). These derive from either self-peptide, primarily from normal cellular turnover, or from proteins of intracellular pathogens, in this case providing a target for recognition by CD8 T cells.

2.2. Class Ib MHC

The study of MHC in regards to the immune system has largely focused on the class Ia molecules. Indeed, it was the study of class Ia MHC that identified the mechanisms by which they are recognised by CD8 T cells, a Nobel Prize winning discovery (Zinkernagel and Doherty, 1974). However, another set of class I MHC genes exists which are termed class Ib MHC, or non-classical MHC. The importance of these molecules to immune development is underscored by their presence and conservation across many species (Shiina et al., 2017; Dai et al., 2018).

Class Ib MHC members include HLA-E, HLA-F and HLA-G in humans (Braud et al., 1999), gene products from the H2-M, H2-T and H2-Q families in mice (Kumanovics and Fischer Lindahl, 2004) (Fig. 2) and the C/E and M regions of the rat (Naper et al., 1995). Structurally, class Ia and Ib molecules are very similar but there are several differences in other areas of their biology that set them apart. While class Ia MHC are highly polymorphic, there is a tendency for some class Ib molecules to have limited polymorphism, influencing the types of antigens they can present to the immune system (Hofstetter et al., 2011). Furthermore, class Ib MHC can exhibit restricted tissue distribution (Ohtsuka et al., 2008), alterations to expression pattern with age (Melo-Lima et al., 2014) and can provide instruction to immune cells across the innate and adaptive immune system (Braud et al., 1999). The importance of class Ib MHC to NK cell biology is underscored by observations using mice deficient for all class I MHC ($\beta2m^{-/-}$) and comparing these to mice in which a specific deletion of class Ia MHC ($K^b/D^{b-/-}$) has been performed. β2m deficient mice support the concept of licensing as NK cells in these animals are hypo-responsive, owing to the complete absence of class I MHC (Sun and Lanier, 2008). However, in class Ia deficient mice, NK cells retain their cytotoxic potential, suggesting a process of licensing dependent on class Ib MHC (Vugmeyster et al., 1998). Thus, class Ib MHC are a specialized family of molecules that play an important role in NK cell biology.

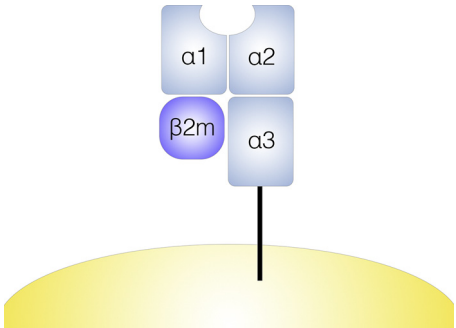

Fig. 1. Schematic representation of the major histocompatibility complex class I molecule (MHC class I). MHC class I molecules exist as heterodimers consisting of three alpha domains (α1, α2 and α3) non-covalently associated with β2-microglobulin (β2m). The α1 and α2 domain form the peptide-binding cleft, whereas the transmembrane region of the α3 domain is responsible for anchoring of the MHC molecule to the cell surface.

2.3. MHC-I interacting NK cell receptors

NK cells have germline-encoded receptors for class I MHC and these are separated into 2 broad groups: the immunoglobulin-like and lectin-like receptors. Receptors from the immunoglobulin-like family are termed Killer cell Immunoglobulin Receptors (KIR) in humans, whereas their functional counterpart in mice, the Ly49 receptors, belong to the lectin-like superfamily. These are the principal receptors by which class I MHC influence the NK cell immune responses. Ly49/KIR molecules exist in either inhibitory or activating forms and share the same basic function that has allowed study of Ly49 to guide our understanding of KIR (Pegram et al., 2011). As these receptors are not subject to the recombination events present in T cells, they exist as polymorphic variants that, in concert with the polymorphism of class Ia MHC, is meant to ensure that each NK cell expresses at least one receptor that facilitates instruction by class I MHC (Pegram et al., 2011). Engagement of class I MHC by the inhibitory receptors transduces inhibitory signals to NK cells and limits immune activation.

2.4. CD94-NKG2 binding

Interestingly, a highly conserved class Ib MHC (H2-T23/Qa-1b or HLA-E, discussed below) also plays a role in signalling the presence of other class I MHC molecules via the presentation of a single, TAP dependent peptide termed the Qa-1 determinant modifier (Qdm) (Aldrich et al., 1992). Qdm peptide comprises of the nonamer AMAPRTLLL (Aldrich et al., 1994) which is derived from the signal sequence of class Ia and some class Ib MHC-I (DeCloux et al., 1997). Therefore, if a cell down-regulates the machinery involved with class Ia MHC biosynthesis, which frequently happens in response to microbial infection or transformation, this loss prevents the pool of Qdm peptides from being generated, subsequently limiting the expression of H2-T23/Qa-1b or HLA-E at the cell surface. The consequence of this down-regulation is loss of inhibitory signals provided by a major family of lectin-like receptors that are conserved between mice and humans, the CD94-NKG2 family (Houchins et al., 1991; Berg et al., 1998; Lanier et al., 1998). CD94-NKG2 receptors are similar to the Ly49/KIR family in that they contain both activating and inhibitory receptors, however they are lectin-like type II integral membrane proteins and exist as disulphide-linked heterodimers (Chang et al., 1995). CD94 lacks a functional cytoplasmic tail, but is necessary to stabilise NKG2 receptors on the cell surface (Brooks et al., 1997). In line with its capacity to inhibit NK cell responses, NKG2A (and its splice variant NKG2B) contains an immunoreceptor tyrosine-based inhibition motif (ITIM) within its cytoplasmic domain which, following interaction with its receptor, results in phosphorylation and recruitment of SHP resulting in inhibitory signalling (Long, 2008). In contrast, NKG2C lacks this ITIM domain but is able to associate with the DAP12 dimer via a lysine residue in the NKG2C transmembrane domain (Lanier et al., 1998). This association provides an activating signal via ITAM motifs in the DAP-12 dimer, with the subsequent tyrosine kinase pathway providing positive regulation (Kumanovics and Fischer Lindahl, 2004).

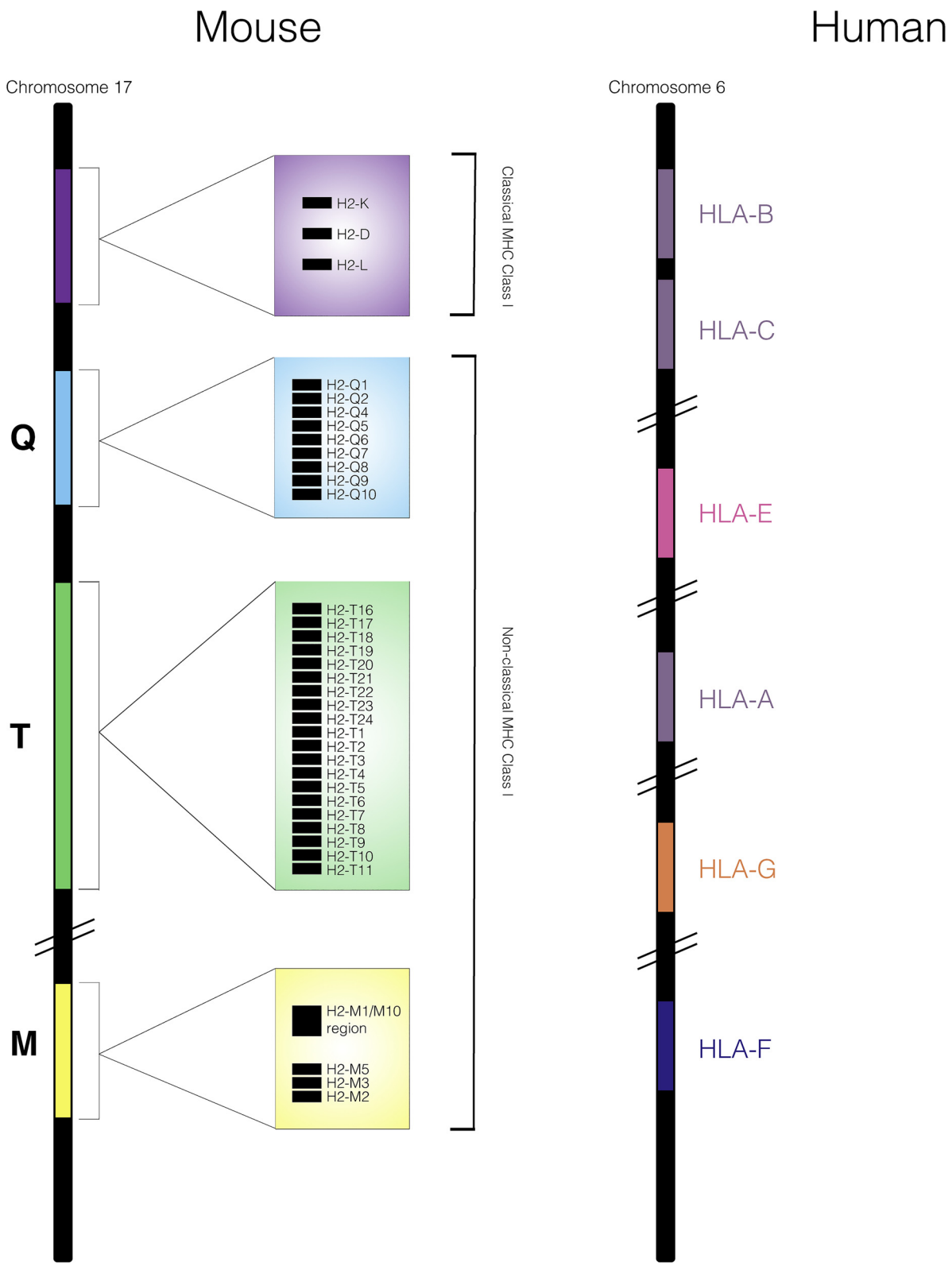

Fig. 2. A. Murine class Ia and class Ib MHC loci on mouse chromosome 17 with MHC class I families being localised in 'clusters' (not to scale). The class Ia MHC region, comprising the H2-K, H2-D and H2-L encoding genes, are followed by Q (blue), T (green) and M (yellow) family members, respectively. B. Human class 1a and class 1b MHC loci on chromosome 5 (not to scale). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

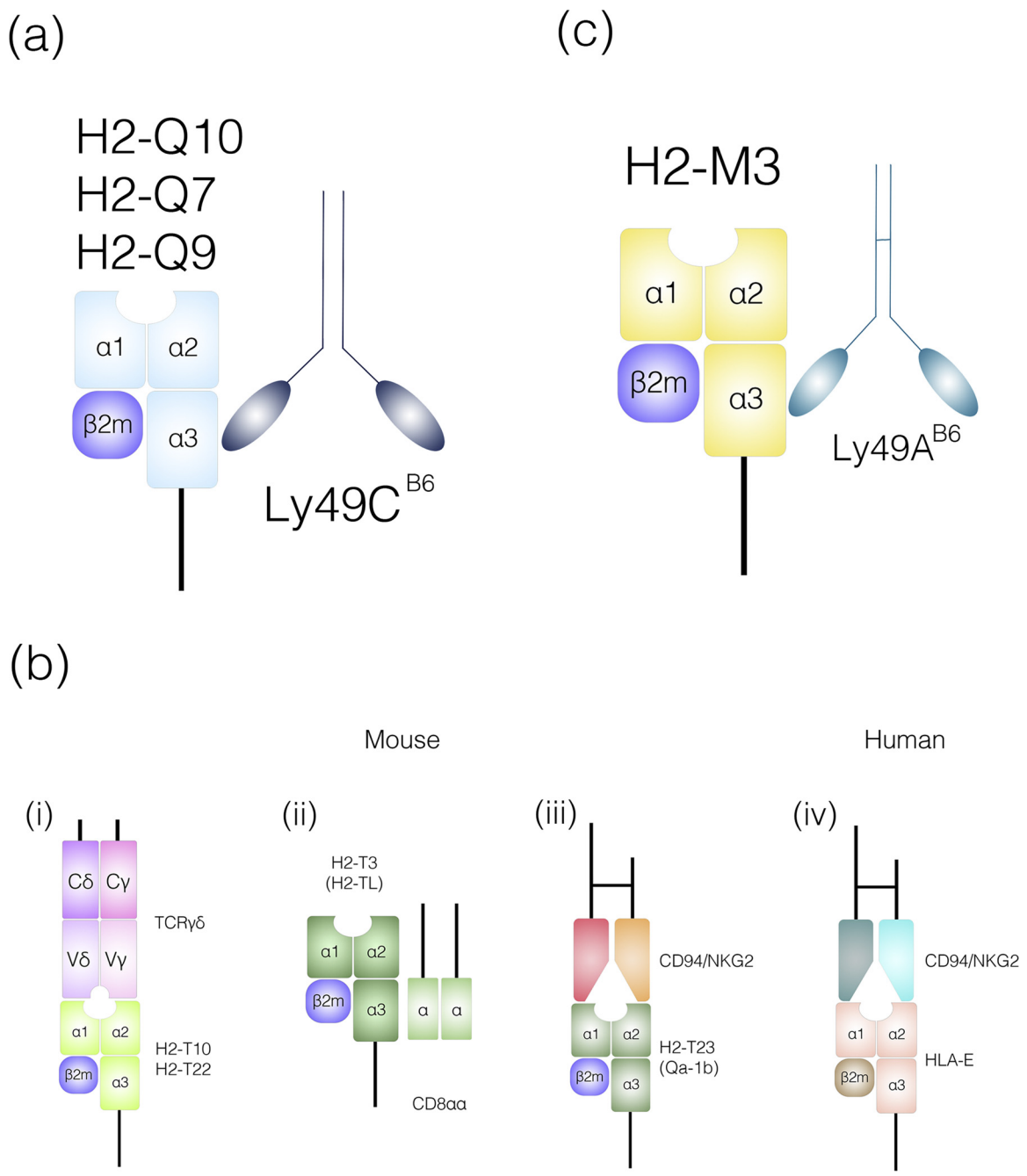

Fig. 3. A. Binding interactions occur between Q family members (H2-Q10, H2-Q7, H2-Q9) and the NK cell inhibitory receptor, Ly49C^{B6}. **B.** H2-M3 binds to the NK cell inhibitory receptor, Ly49A^{B6}. The binding is shown as for the interaction with Ly49A^{B6} and the classical MHC-I H-2D^{d} (Wang et al., 2002) but as yet the specific binding of H2-M3 to Ly49A^{B6} is yet to be resolved. **C.** Binding interactions of T family members in the mouse and human: **(i)** H2-T10 and H2-T22 binding the T cell receptor on gamma delta T cells (TCRγδ), **(ii)** H2-T3 (H2-TL protein) binds the CD8αα homodimer through its α3 domain, **(iii)** H2-T23 (Qa-1b protein) binds to the CD94/NKG2 complex, which elicits activatory or inhibitory signals depending on the nature of the NKG2 polypeptide, **(iv)** HLA-E, the hypothesised human homologue of H2-T23/Qa-1b, is able to bind the CD94/NKG2 complex.

While these patterns of recognition have been informative, they are incomplete as many of the Ly49 receptors do not recognise class Ia MHC (Hanke et al., 1999). This provided a paradox for NK cell immunologists as it was obvious that NK cells with 'orphan' Ly49 receptors did exist but they did not spontaneously activate at any time (Dorfman and Raulet, 1996). However, the identification of class Ib MHC interacting with NK cell receptors provided a potential resolution of this phenomenon. Therefore, this review aims to summarise what is known about murine class Ib MHC, their interacting receptors on NK cells, and how they play an instrumental role in shaping the immune system.

3. H2-Q family

Very little is known about the majority of Q family MHC, mainly due to the lack of available reagents specific for these molecules. Apart from genetic identification, there has been little research on the biological function of H2-Q1, -Q2, -Q3 and –Q4. A potential biological role for H2-Q5 was described following the observation that it was capable of donating Qdm to Qa-1b (Renthal et al., 2011). H2-Q5 is expressed at high levels in the immune-privileged brain, in which class Ia MHC-I are rarely found, and it is here that it acts to donate Qdm, which cannot be generated from other sources (Renthal et al., 2011).

Extensive research into two members of the Q family has allowed the identification that they can recognise and bind Ly49 receptors. These are H2-Q10 (encoded by the gene *H2-Q10*) and Qa-2 (encoded by the almost identical *H2-Q7/9* or *Q6/8* depending on strain – alternatively named the *PED* gene (Wu et al., 1998). Qa-2 is the best studied Q family protein, and was one of the first class Ib members characterised at a protein level (Sullivan and Flaherty, 1979). Qa-2 can be expressed as one of two splice variants, one being a non-soluble, membrane-bound 40kDa GPI-linked surface variant, found in resting T lymphocytes and some tumours. However, upon activation of T cells or advancement of tumour burden, the levels of Qa-2 are significantly increased with Qa-2 expressed primarily as a 39kDa soluble splice variant that is secreted from the cell (Soloski et al., 1986). It has been proposed that the soluble Qa-2 is directly derived from the intracellular product, without having to be expressed as the membrane bound form first (Einhorn et al., 1991).

Similar to HLA-G, which is highly expressed by extravillous trophoblasts and is believed to be important in the maintenance of pregnancy (Chumbley et al., 1993), Qa-2 has been best defined in embryonic development, as it promotes embryonic cleavage division, birth weight, litter size and weaning weight of mouse litters (Chumbley et al., 1993; Warner et al., 1991; Warner et al., 1993). These identifications played a role in suggesting that Qa-2 represents the murine homologue of HLA-G (Warner et al., 1993; Comiskey et al., 2003). However, this seems unlikely given the significant differences in the biology of Qa-2 and HLA-G. For example, HLA-G expression is largely restricted to extravillous trophoblast cells (Kovats et al., 1990) while Qa-2 has a much broader expression pattern (Ohtsuka et al., 2008). Further complicating the hunt for a HLA-G homologue in mice is the identification that H2-Q5 can also be found in the ovary and developing placenta (Ohtsuka et al., 2008) and, like HLA-G, is expressed in multiple spliced isoforms (Renthal et al., 2011).

These differences not withstanding, Qa-2 represents and interesting class Ib MHC as it affects components of the immune system in differing ways. In the case of tumours controlled by cytotoxic T cells, studies into Qa-2 expression are not clear, although current research tends to report anti-tumorigenic properties of Qa-2. In the case of 4T1 breast cancer (da Silva et al., 2017a), B16 melanoma (Chiang and Stroynowski, 2004) and Ehrlich tumours (da Silva et al., 2017b), all of which are controlled by cytotoxic T lymphocytes (CTLs), Qa-2 expression is positively correlated with tumour clearance. However, there are also two conflicting studies by the same group examining Qa-2 in NK-cell sensitive tumours. Using the melanoma line B78H1, Chiang and colleagues found that presence of Qa-2 inhibited the ability of NK cells to kill the melanoma cells (Chiang et al., 2002). This is in accordance to expectations that Qa-2 is acting in a similar way to the class Ia MHC-I and its presence inhibits NK-cell killing via binding to an inhibitory NK-cell receptor. However, a year later, the same group published another report on the same melanoma cell line in the mouse, now suggesting that Qa-2 expression in the B78H1 cells actually leads to enhanced tumour killing by CTLs and NK cells (Chiang et al., 2003). Therefore, the role of Qa-2 in tumour growth and progression is unclear, at least in the case of NK-cell sensitive tumours. However, recent studies from our laboratory have demonstrated that Qa-2 is capable of binding to Ly49C (Fig. 3a), supporting the observations that Qa-2 expression on NK cell sensitive tumours prevents killing.

The other relatively well characterised Q family member is H2-Q10. Similar to Qa-2, H2-Q10 is a soluble molecule, however H2-Q10 is soluble owing to truncations in the transmembrane and cytoplasmic region, unlike Qa-2, whose solubility is controlled by GPI linkage (Kress et al., 1983). While Qa-2 is ubiquitously expressed across all strains of mice, H2-Q10 transcript levels across different strains of mice was only detected in the liver of adult mice (Cosman et al., 1982). Due to the soluble nature of H2-Q10, it is also found in the serum at very high concentrations – between 10 - 60μg/ml (Lew et al., 1986). Interestingly, H2-Q10 demonstrates differential expression during development as it is detectable in the thymus and intestine of neonatal mice but in relatively low levels in the liver (Melo-Lima et al., 2014). However, as the mice age, H2-Q10 expression is completely restricted to the liver, with transcript levels increasing with age until at least 60 days after birth (Melo-Lima et al., 2014; Cosman et al., 1982). Significantly, H2-Q10 expression spikes around the point of weaning at much the same time that changes to the immune cell repertoire of the liver also change (Andrews and Smyth, 2010). Much like Qa-2, H2-Q10 presents a classical peptide repertoire like those observed in the class Ia MHC (Zappacosta et al., 2000), an observation reinforced with the solving of the crystal structure of H2-Q10 (Sullivan et al., 2016).

The biological relevance of the solubility of H2-Q10 has been questioned since its identification. In 1987, Mann and colleagues determined that despite high levels of H2-Q10 in the serum, if Q10 is expressed as an artificial membrane-bound molecule, there is specific CTL activity generated against H2-Q10. This indicated that the soluble form is not tolerogenic, at least in the case of CTLs (Mann et al., 1987) but a potential role for H2-Q10 in NK cell responses was not identified until 2016 (Sullivan et al., 2016). Tetramer generation facilitated the identification of Ly49C as a receptor for H2-Q10 (Sullivan et al., 2016) (Fig. 3a). This suggested that, at least in the context of NK cells, H2-Q10 may act to regulate NK cell activation through the interaction with an inhibitory Ly49 receptor but this function is yet to be demonstrated. Although not yet directly proven, it is expected from its sequence, that H2-Q10 also functions as a Qdm donor in the liver, further reinforcing its central role in liver NK cell biology.

In light of the recent identifications that Qa-2 and H2-Q10 bind to Ly49C (Sullivan et al., 2016) coupled with sequence identity and presentation of a polymorphic peptide repertoire (Zappacosta et al., 2000; Sullivan et al., 2016; He et al., 2001), it is becoming apparent is that there are significant biological, structural and functional similarities between the Q family and class Ia MHC. This suggests that the Q family is closely related to the class Ia MHC and further study of these genes may shed light on the evolution of the NK cell receptor repertoire.

4. H2-T family

The most comprehensively studied class Ib MHC is the non-polymorphic molecule Qa-1b (H2-T23), the murine homologue of HLA-E (Braud et al., 1998), which binds to CD94/NKG2A/B, 2C and -2E heterodimers found on innate and adaptive subsets of lymphocytes. The importance of this molecule to immunity is highlighted by the observation that almost every species of animal carries a functional or positional homologue of this gene associated with similar conservation of CD94/NKG2 (Kurepa and Forman, 1997). Unlike many other members of the class Ib MHC family, Qa-1b and HLA-E exhibit expression in most tissues, albeit at lower levels (Ohtsuka et al., 2008; Wei and Orr, 1990). It is the conservation of Qa-1b and HLA-E between species and wide spread expression pattern that highlights the evolutionary importance of Qa-1b/HLA-E axis in immunity.

The Qa-1b peptide binding groove is specialised such that a significant dominance of Qdm peptide is presented (DeCloux et al., 1997). The crystal structure of Qa-1b provides a structural basis for the restricted peptide-binding, showing that Qa-1b has 5 hydrophobic pockets that anchor the Qdm peptide (Zeng et al., 2012). Similar to its murine counterpart, HLA-E peptide binding is highly restricted, favouring a nonamer peptide derived from the leader sequence of MHC-I, and tolerates only minor variations at positions 2, 7 and 8 (O'Callaghan and Bell, 1998). Moreover, despite the sequence variation between Qa-1b and HLA-E the conformation of the peptide bound by each molecule was remarkably similar (Zeng et al., 2012). Further reinforcing the importance of Qa-1b/HLA-E like molecules during the evolution of immunity, a comparison of the ability of leader sequences from evolutionarily diverse organisms to bind to Qa-1b found that, albeit with less affinity than Qdm, they were all still bound and presented (Kurepa and Forman, 1997).

Table 1
Summary of class Ib MHC family members, their peptide-presentation status, known binding partners and any identified human homologues.

GENE NAME	PROTEIN NAME	PEPTIDE PRESENTATION	INTERACTING RECEPTOR	INTERACTING CELL	HUMAN HOMOLOGUE
H2-Q10	H2-Q10	Yes	Ly49C^{B6}	NK cell	Unknown
H2-Q5	H2-Q5	No	Unknown	Unknown	Unknown
H2-Q7/9	Qa-2	Yes	Ly49C^{B6}	NK cell	Unknown
H2-T23	Qa-1b	Yes	CD94-NKG2A/C/E	NK cell	HLA-E
H2-T3	H2-TL	No	CD8αα homodimers	CD8+ T cell/IEL	Unknown
H2-T10	H2-T10	No	γδ TCR (G8 and KN6)	γδ T cell	Unknown
H2-T22	H2-T22	No	γδ TCR (G8 and KN6)	γδ T cell	Unknown
H2-M3	H2-M3/HMT	Yes	Ly49A^{B6}, αβ TCR	NK cell	None

Having identified the peptide, it became possible to elucidate the target of HLA-E/Qa-1b using fluorescently labelled tetramers. These studies demonstrated that Qa-1b was recognised by CD3$^-$/NK1.1$^+$ splenocytes independently of Ly49 inhibitory receptors (Salcedo et al., 1998). The NK cell receptor that recognises Qa-1b was discovered in 1998 as the inhibitory receptor heterodimer CD94/NKG2A, followed up by the determination that the activating family members CD94/NKG2C/E also recognise Qa-1b (Vance et al., 1998) (Fig. 3c). Similarly, HLA-E tetramers bound to cells transduced with CD94/NKG2A, and –C receptors, but not KIRs (Braud et al., 1998). The significance of this interaction was outlined by the observation that HLA-E on target cells was sufficient to prevent lysis by CD94/NKG2A$^+$ clones (Borrego et al., 1998). The structure of HLA-E in complex with CD94-NKG2A has been solved (Petrie et al., 2008) and showed that the CD94 subunit dominated the contacts with HLA-E, whereas the NKG2A subunit was more peripheral to the interaction, indicating the primary role of CD94 was to contact HLA-E whereas the NKG2 unit dictated signalling.

Under homeostatic conditions, a large proportion of NK cells express NKG2A and hence are negatively regulated via the presence of Qa-1b/HLA-E. However, in instances where MHC-I is down-regulated or antigen processing altered (such as following viral infection), this perturbs the presentation of Qdm and can result in NK cell activation through lack of NKG2A signalling. For example, human cytomegalovirus (HCMV) expresses a suite of immune evasion molecules that disrupt MHC-I expression. However, one such molecule, HCMV UL40, also directly targets the interaction between HLA-E and CD94-NKG2A. Ulbrecht and colleagues determined that UL40 which contains a peptide identical to the peptide derived from the leader-sequence of many MHC-I that binds to HLA-E (Ulbrecht et al., 2000). This provides HCMV with the ability to inhibit class I expression and evade CD8 T cells, while also ensuring continued expression of HLA-E as a means to evade NK cells. However, there is also strong correlation between the frequency of activating NKG2C(+) NK cells and positive serology for HCMV (Guma et al., 2004), indicating a positive role for HLA-E in controlling HCMV infection. Thus, there appears to be a highly dynamic, evolutionary arms race between HCMV and the immune system with attack and counter strategies generating utilising the HLA-E/NKG2 axis.

Current data suggests that other members of the T family of MHC are more closely associated with γδ and CD8αα T cells (Ito et al., 1990; Wingren et al., 2000; Crowley et al., 1997). However, phylogenetic studies have demonstrated that H2-T5, -T7 and –T13 are more closely related to the Q family and class Ia MHC than other members of the T family (Hughes and Nei, 1989). Significantly, H2-T13 (otherwise referred to as blastocyst MHC) has been shown to act as a negative regulator of NK cells, using a mechanism that is only partly dependent upon Qa-1b (Tajima et al., 2003) (Fig. 3c). It is also worth noting that H2-T13 expression occurs at the blastocyst stage and is found in the placenta, meaning that, like Qa-2 and H2-Q5, it too has been proposed as a homologue for HLA-G (Sipes et al., 1996). Thus, the capacity for members of the T family to act directly on NK cells requires further investigation, particularly in light of the observation that many of these genes show developmental expression in the embryo and placenta (Ohtsuka et al., 2008).

5. H2-M family

The H2-M family is the least studied of all class Ib MHC, with the only functional data being provided through studies of H2-M3. While there doesn't seem to be a direct homologue between humans and mice, the M family may have arisen due to a gene duplication event that has evolved to complement the important functions of the other class Ib MHC-I in an organ or cell-specific manner.

H2-M3 was initially named the MHC-I heavy chain of the maternally transmitted antigen (HMT). As its name suggests, H2-M3 is maternally transmitted, and is found on both stimulated and unstimulated T and B lymphocytes, as well as several tumour lines (Fischer Lindahl et al., 1980). H2-M3 has anchor residues at positions 34 (Q), 167 (W) and 171 (F) in the H2-M3 heavy chain, which separates H2-M3 from all the other class I molecules, as it enforces the preferential presentation of *N*-formylated peptides. Thus, H2-M3 has evolved to present peptides that originate from either the mitochondria or invading bacteria (Smith et al., 1994; Morse et al., 1996). The structure of H2-M3 binding a formylated nonamer peptide fMYFINILTL was solved in 1995 and this confirmed that the unique binding groove of H2-M3 has a closed A pocket, leading to the necessity of *N*-formylation of the peptide (Wang et al., 1995). In addition, due to the shortened groove, H2-M3 preferentially presents heptameric or hexameric peptides (Dabhi and Lindahl, 1998). H2-M3 targeting by cytotoxic T cells has been best studied by H2-M3 presenting a peptide from laboratory strain of *Listeria monocytogenes*, with an identification of H2-M3 binding and presenting the hexapeptide fMIGWII, or the pentapeptide fMIVIL, targeting the cell for lysis by CD8+ cytotoxic T cells (Lenz et al., 1996; Gulden et al., 1996; Princiotta et al., 1998). From an intracellular source, H2-M3 also has been characterised in presenting the mitochondrially derived NADH dehydrogenase subunit 1 (ND1) (Berg et al., 1999). Interestingly, although H2-M3 preferentially binds *N*-formylated peptides, it does have the ability to present non-formylated peptides, as shown by Byers and colleagues with H2-M3 presenting a viral epitope from the influenza virus, leading to selective lysis by CTLs in-vitro (Byers and Fischer Lindahl, 1998).

H2-M3 is retained intracellularly in the golgi or the ER in the absence of peptide, and is only trafficked to the cell surface upon receipt of peptide in a TAP dependent manner, most efficiently on antigen-presenting cells (Chiu et al., 1999). The kinetics of this presentation is similar to that of the class Ia MHC I receptors, and therefore having a pool of empty H2-M3 awaiting peptide means that it can respond exquisitely well to bacterial infections (Chiu et al., 1999).

However, a relatively recent paper discovered that H2-M3 was seen to have an exceptionally important role in not only a CD8+ T cell response, but also in NK cell licensing and activation. Until this identification, NK cell licensing was attributed only to inhibitory NK cell receptors binding to class Ia MHC-I in the 'at-least-one' hypothesis (Held et al., 1996; Valiante et al., 1997). However, in 2012, it was found that the inhibitory NK cell receptor Ly49A could bind to H2-M3 (Fig. 3b), leading to licensing of the NK cell, a process essential for optimal

cytotoxic potential and cytokine producing capacity (Andrews et al., 2012). This was particularly interesting, as before this discovery, Ly49A was an 'orphan' receptor with no known interacting partner. This suggests that the class Ib MHC played a more significant role in Ly49 mediated instruction than previously recognized and that this family of molecules could be the missing link to other orphaned Ly49 receptors.

6. Conclusion

Class Ib MHC are a diverse family of proteins that play an important role in immune responses through interactions with NK, γδT or $CD8^+$ T cells (Table 1). These interactions target pathological events including cancer and infection. Some members of the class Ib MHC appear to be the primary MHC interaction in immune-privileged organs while other members outcompete class Ia MHC receptor binding, indicating that they are the primary ligand in those settings. It has also become apparent that class Ib MHC are varied in their degree of evolution away from the class Ia MHC-I. Similar to what has been demonstrated in mice (Sullivan et al., 2016; Andrews et al., 2012), recent evidence has demonstrated that the rat class Ib MHC represents an important target for activating and inhibitory members of the rat Ly49 family (Dai et al., 2018). Clearly, the class Ib MHC are crucial in shaping the immune response, and further studies are critical to fully elucidate these mechanisms.

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