EMBO
reports

review

Non-classical major histocompatibility complex proteins as determinants of tumour immunosurveillance

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Tumours develop in vertebrate organisms endowed with immune systems that are potentially able to eradicate them. Nevertheless, our ever-increasing understanding of the complex interactions between lymphocytes and tumour cells fuels the long-standing hope of developing efficient immunotherapies against cancer. This review focuses on a versatile family of proteins, the major histocompatibility complex class Ib, which has been recently implicated in both the establishment of anti-tumour immune responses and in tumour immune response evasion. We focus on a subset of class Ib proteins, human leukocyte antigen (HLA)-G, Qa-2, CD1d and NKG2D ligands, which bind to either stimulatory or inhibitory receptors expressed on T, natural killer (NK) and NKT lymphocytes, and thereby modulate their anti-tumour activity.

Keywords: MHC; cancer; innate; lymphocyte; natural killer

EMBO *reports* (2007) **8,** 1024–1030. doi:10.1038/sj.embor.7401090

Introduction

Paul Ehrlich's discoveries 100 years ago seduced immunologists with the idea that cytotoxic cells are able to eliminate tumours, much like they destroy virus-infected cells. In the 1950s, MacFarlane Burnet proposed that the immune system surveys cellular transformation and prevents the development of tumours (Burnet, 1957). This 'tumour surveillance hypothesis' has prompted the identification of a panoply of tumour-specific or tumour-enriched peptides over the past 20 years. These peptides are presumably the antigens presented by classical major histocompatibility complex (MHC) molecules to the cytotoxic T lymphocytes (CTL) of the adaptive immune system, particularly T-cell receptor (TCR) $\alpha\beta^+$ $CD8^+$ cells. Although their identification allowed the development of tumour vaccines—which are being assayed in clinical trials—the overall success of such immunotherapeutic strategies has been limited by the common downregulation of such antigens and/or their MHC class Ia presentation elements in advanced tumours. However, cancer immune surveillance is likely to involve both the adaptive and the innate immune systems, as is true for host immunity to pathogens. Indeed, many reports have illustrated the capacity of innate lymphocytes, γδ T, natural killer (NK) and NKT cells, to detect and destroy tumour cells independently of classical MHC presentation (Girardi *et al*, 2001, 2003; Smyth *et al*, 2001; Street *et al*, 2004).

The molecular mechanisms responsible for the immune recognition of tumour cells are still the subject of much debate. However, recent data has highlighted the many roles played by members of a plastic protein family: the non-classical or class Ib MHC (MHC Ib). MHC Ib molecules are structurally related to class Ia proteins because they show a typical (α1–α2) MHC fold on a single polypeptide, which, in the case of Ib, does not pair up obligatorily with β2-microglobulin (Rodgers & Cook, 2005). Furthermore, although many MHC Ib genes are also located in the MHC locus, they tend to be oligomorphic—few alleles exist in the population—which is in marked contrast with the extensive polymorphism of class Ia. The oligomorphism of class Ib might therefore be an advantage for the design of cancer therapies with a wider application within the cancer patient population. Amino-acid sequence identity/homology is not a useful criterion to establish relationships between class Ib members, as functional equivalents across species—for example, proteins that bind to a given receptor—are often not orthologous. Some class Ib genes are in fact more closely related to class Ia genes in evolutionary terms than to other class Ib members (Rodgers & Cook, 2005). MHC Ib proteins have been largely shown to bind to stimulatory or inhibitory receptors expressed on T, NK and/or NKT lymphocytes (Fig 1); there are, however, a few exceptions such as the haemochromatosis antigen (HFE) or the neonatal Fc receptor (FcRn).

Here, we focus on the ability of some MHC Ib proteins to act as 'reporters' of cellular transformation and trigger anti-tumour immune responses. We have selected three examples to illustrate the various mechanisms by which MHC Ib can activate or inhibit anti-tumour lymphocytes.

Qa-2 and HLA-G present peptides to $CD8^+$ T cells

Murine Qa-2 and human leukocyte antigen (HLA)-G are 'young' MHC Ib proteins in that they diverged from MHC Ia less than 20

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Submitted 30 January 2007; accepted 13 September 2007

million years ago, and therefore have many similarities with the classical Ia molecules: they present a repertoire of nonameric peptides, contain a CD8 binding loop, associate with β2-microglobulin (β2m), display similar exon–intron organization and even share a high degree of amino-acid sequence homology (Seliger *et al*, 2003).

The murine Qa-2 region, located in the H2-Q class Ib locus, encodes the proteins Q8 and Q9 in C57BL/6 (B6) mice, which differ from each other by 20 amino acids located in the α1 and α2 domains. These proteins have overlapping peptide-binding motifs and are recognized by cross-reactive anti-tumour CTLs, and therefore appear to be functionally equivalent (Chiang & Stroynowski, 2006). Surface expression of Qa-2 molecules requires a functional TAP (transporter associated with antigen processing) peptide transporter (Tabaczewski *et al*, 1994) and is dependent on ERAP1 (endoplasmic-reticulum-associated peptidase 1) processing, suggesting that peptides loaded onto Qa-2 molecules are generated through the conventional class Ia antigen-processing pathway (Yan *et al*, 2006). Qa-2 transcripts are widely expressed at low levels in healthy tissues, both in haematopoietic and in non-haematopoietic cell types (Ungchusri *et al*, 2001). However, Q9 expression was found to be lost or severely reduced in a large panel of *in vivo*-derived tumour cell lines, including cells derived from T- and B-cell lymphoma, mastocytoma, melanoma and hepatoma (Ungchusri *et al*, 2001). Serological and reverse-transcriptase-PCR (RT-PCR) analyses have also shown that the primary B16 melanoma tumour cell line and all its variants had no Q8 and Q9 expression, whereas the control melanocyte line was positive for these antigens (Chiang *et al*, 2003). These results suggest that Q8 and Q9 expression is silenced early during tumour progression.

Several *in vitro* and *in vivo* studies have nonetheless shown Q9 involvement in tumour rejection. Syngeneic B6 mice injected with the B78H1 melanoma cell line—selectively devoid of TAP2 and class Ia (Kb and Db) transcripts and genetically manipulated to re-express Q9 on its surface—were protected from melanoma outgrowth, unlike mice challenged with class Ia-negative B78H1 or with TAP2-transfected parental melanoma cells (Chiang *et al*, 2003). Subsequent studies have shown $CD8^+$ CTL involvement in Q9-mediated protection from melanoma development, as protection is lost in CD8 knockout and severe combined immunodeficiency (SCID) mice, but not in CD4 knockouts (Chiang & Stroynowski, 2004). Furthermore, it was demonstrated that mice surviving the original challenge became resistant to subsequent doses of Q9-positive melanoma, suggesting that Qa-2 acted as a restriction element for anti-tumour $CD8^+$ T cells, and that both effector and memory cells were generated in immunized mice (Chiang & Stroynowski, 2004). Q9-restricted CTL responses able to recognize lung carcinoma and T-cell lymphoma have also been observed (Chiang & Stroynowski, 2005). In addition, Q9 was shown to act as a restriction element for a tumour antigen common to these tumours and melanoma. It was observed that CTL clones raised in response to a challenge with Q9-expressing 3LLA9F1 lung carcinoma, RMA T-cell lymphoma or GMQ9TAP B78H1 melanoma efficiently killed all of these tumours in cytotoxicity assays in a Q9-restricted manner. This also suggests that CTLs generated in the primary response establish a pool of memory cells that exhibit cross-reactivity against various tumours (Chiang & Stroynowski, 2005). Qa-2 proteins are also recognized by NK lymphocytes, and these have been shown to be essential for the rejection of large doses of Q9-positive melanoma cells even in the presence of a CTL response (Chiang *et al*, 2003). Therefore, it will be important to analyse the interplay between $CD8^+$ T and NK cells in Qa-2-dependent anti-tumour responses.

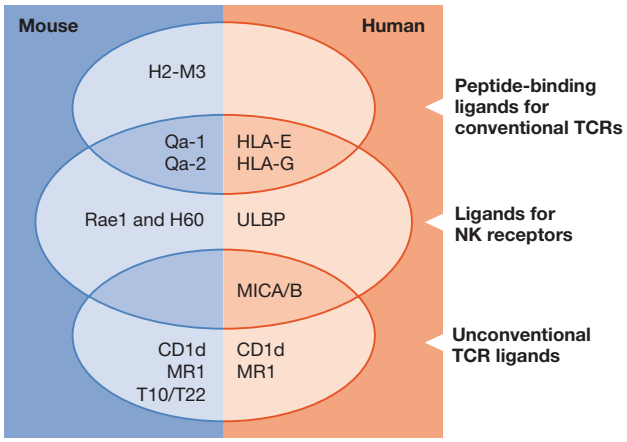

Fig 1 | Major histocompatibility complex class Ib proteins bind various immunoreceptors. Listed are major histocompatibility complex class Ib (MHC Ib) molecules for which receptor binding has been well characterized. Conventional T-cell receptors (TCRs) are those of polyclonal αβ T cells; unconventional TCRs correspond to oligoclonal T-cell subsets, such as natural killer (NK) T cells (for CD1d), γδ T cells (T10/T22, MICA/B) or gut-associated T cells (MR1). Proposed functional homologues between mice and humans are in the same row. H60, histocompatibility antigen 60; HLA, human leukocyte antigen; MICA/B, MHC class I chain-related peptides A/B; Rae1, retinoic acid early inducible gene 1; ULBP, UL16 binding protein.

Although there is no human orthologue of Qa-2, some have suggested HLA-G is its functional homologue as both proteins have immunoregulatory roles, are involved in embryonic development, and exist in membrane-bound and soluble forms that arise by alternative splicing (Comiskey *et al*, 2003). HLA-G expression is augmented in various tumours, including melanomas, breast, renal, ovarian and lung carcinomas, gliomas, B and T non-Hodgkin lymphomas, acute leukaemias and colorectal cancers (Rouas-Freiss *et al*, 2005). However, it is important to note that HLA-G expression is not detected in all tumours; for example, HLA-G expression has been reported in ocular tumours such as retinoblastoma, whereas it has not been detected in uveal melanomas, even after treatment with interferon-γ (IFN-γ; Hurks *et al*, 2001). *HLA-G* expression is highly inducible and cytokines, such as interleukin (IL)-10, leukaemia inhibitory factor (LIF), tumour necrosis factor-α (TNF-α) and IFN-γ, differentially regulate its transcription in several tumour cell lines (Carosella & Dausset, 2003). Stress-inducing factors, such as heat shock and arsenite treatment, also induce *HLA-G* transcription in tumour cells (Ibrahim *et al*, 2000). Furthermore, it was recently shown that *HLA-G* expression is regulated by hypoxia in a hypoxia-inducible factor 1 (HIF1)-dependent manner (Mouillot *et al*, 2007). HLA-G1 protein expression can also be controlled at the post-translational level because its levels on the cell surface of carcinoma and melanoma cells are reduced upon activation of the NF-κB signalling pathway (Zidi *et al*, 2006). Unlike HLA class I genes, *HLA-G* expression is controlled by *cis*-acting epigenetic

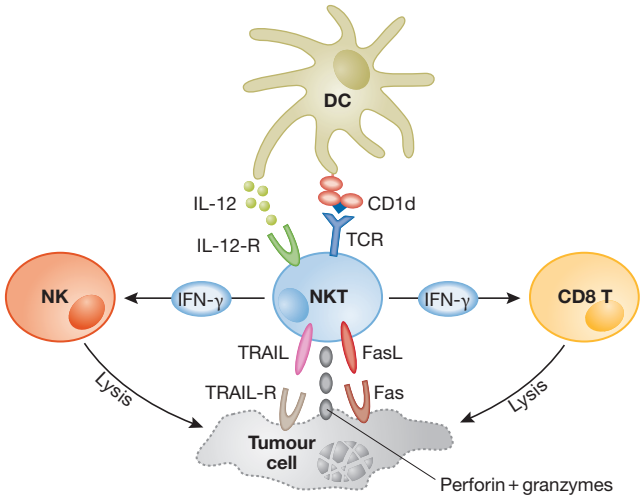

Fig 2 | Anti-tumour responses mediated by CD1d-activated type I natural killer T cells. After receiving activation signals from dendritic cells (DCs)—through CD1d and IL-12—NKT cells can either lyse tumour cells directly using the perforin/granzyme system or ligands for death receptors, or stimulate other cytotoxic cells such as NK and CD8$^+$ T cells through IFN-γ secretion. IFN-γ, interferon-γ; IL-12, interleukin 12; NK, natural killer; NKT, natural killer T cell; TCR, T-cell receptor; TRAIL, tumour necrosis factor-related apoptosis inducing ligand.

mechanisms, such as DNA methylation/demethylation and histone deacetylation/acetylation (Chang *et al*, 2003; Guillaudeux *et al*, 1995; Mouillot *et al*, 2005). For example, when cells of HLA-G positive melanoma tumours are subject to long-term *in vitro* culture, their HLA-G expression levels decrease over time, correlating with methylation of the *HLA-G* promoter. This suggests that lack of exposure to tumour microenvironmental conditions might lead to silencing of the gene (Chang *et al*, 2003).

Qa-2 expression in murine tumours makes them more susceptible to CTL-mediated lysis, whereas the aberrant expression of HLA-G antigens by human tumour cells seems to constitute an immune evasion mechanism. This would be analogous with their function in trophoblasts, which is to protect the fetus from maternal immune attack (Carosella *et al*, 2003). Indeed, several lines of evidence have shown that HLA-G can inhibit anti-tumour lymphocytes (Rouas-Freiss *et al*, 2005). First, HLA-G molecules were shown to interact directly with inhibitory receptors present on the surface of T (ILT-2) and NK cells (KIR2DL4, ILT-2), as well as monocytes/macrophages and dendritic cells (ILT-2 and ILT-4). Second, HLA-G molecules stabilize the cell surface expression of other non-classical HLA molecules such as HLA-E, which convey additional suppressive signals to lymphocytes through the widely expressed CD94/NKG2A killing inhibitory receptor. Third, HLA-G1 ligation decreases the secretion of IFN-γ and TNF-α, which are crucial for anti-tumour immunity (Rouas-Freiss *et al*, 2005). Fourth, antigen-presenting cells (APCs) that express HLA-G1 were shown to induce CD4$^+$ T-cell anergy and to trigger their differentiation into suppressor cells that block CTL function (LeMaoult *et al*, 2004). This effect could either be a consequence of the interaction of HLA-G with an unknown receptor expressed by CD4$^+$ T cells, or an inhibitory effect of HLA-G on the APCs themselves. The HLA-G$^+$ APCs present in cancer patients might thus be partly responsible for tumour immune escape owing to their suppressive properties. Furthermore, soluble HLA-G5 has been directly implicated in the inhibition of CD4$^+$ and CD8$^+$ T-cell proliferation. It causes cell cycle arrest by altering the balance between inhibitory molecules and cyclins, inducing the accumulation of $p27^{kip}$ and the reduction of cyclins D2, E, A and B (Bahri *et al*, 2006). Recently, it has also been shown that HLA-G molecules are transferred from tumour cells to activated NK cells by trogocytosis (Caumartin *et al*, 2007), which consists of the rapid transfer of intact cell-surface proteins between cells in contact with each other. The acquisition of HLA-G1 by NK cells blocks their proliferation and cytolytic function owing to its interaction with ILT2 on other NK cells and therefore induces a temporary state of immunosuppression in the NK-cell population (Caumartin *et al*, 2007). Consistent with its immunoevasive role, HLA-G expression in some malignancies, such as colorectal cancers, correlates significantly with increased depth of invasion, histological grade, lymph nodal metastasis and clinical stages of the disease, suggesting that its presence could act as an independent prognostic factor for cancer patients (Ye *et al*, 2007).

Despite the vast amount of data, the role of HLA-G as an inhibitory ligand for anti-tumour lymphocytes remains controversial. For example, the main NK cell receptor for HLA-G, KIR2DL4, can also function as an activating receptor. Indeed, KIR2DL4 engagement has been reported either to inhibit cytotoxicity (Ponte *et al*, 1999) or to promote cytotoxicity and IFN-γ secretion (Kikuchi-Maki *et al*, 2003; Rajagopalan *et al*, 2001). Therefore, the elucidation of the functional consequences of HLA-G ligation, as well as the specific roles of membrane-bound and soluble forms of the protein (Morandi *et al*, 2007), require further investigation.

CD1d presents lipids to NKT cells

CD1d, a conserved member of the small CD1 protein family, presents lipid/glycolipid—instead of peptidic—antigens to TCR αβ complexes expressed by anti-tumour NKT cells in mice and humans (Godfrey *et al*, 2004). The dependence on CD1d for development and activation is the defining characteristic of NKT cells, which also express NK-cell-associated surface markers. NKT cell subsets can be further characterized by their TCR repertoire: type I NKT cells express an invariant TCR (using the Vα14Jα18 gene segments in mice and the Vα24 segment in humans), whereas type II cells display a more diverse repertoire (Godfrey *et al*, 2004). Although NKT cells are endowed with cytolytic factors that eliminate tumour cells such as perforin, Fas-ligand and TRAIL (tumour necrosis factor-related apoptosis inducing ligand) (Kawano *et al*, 1998), they also secrete large amounts of IFN-γ, which, in turn, stimulates anti-tumour NK and CD8$^+$ CTLs (Smyth *et al*, 2002; Fig 2).

CD1d can present various ligands to NKT cells (Zhou *et al*, 2004; Kinjo *et al*, 2005; Mattner *et al*, 2005), including tumour-derived lipids and glycolipids (Brutkiewicz, 2006). For example, the disialoganglioside GD3 is strongly upregulated in some tumours such as melanomas and can be cross-presented by CD1d-expressing murine APCs to NKT cells (Wu *et al*, 2003). Furthermore, some glycolipid fractions of tumour cell membranes are presented by CD1d and activate NKT cells (Gumperz *et al*, 2000). However, it has also been shown that glycolipid shedding by tumour cells can inhibit CD1d-mediated recognition of the tumour target (Sriram *et al*, 2002). Although the repertoire of transformation-induced glycolipids

Table 1 | mRNA/protein expression patterns for NKG2D ligands

NKG2D ligand	Basal	Augmented*
MICA/B (human)	Protein in gastrointestinal epithelium, endothelial cells, fibroblasts; mRNA in keratinocytes Soluble protein in placenta	Protein in melanoma, myeloma, lymphoma, and colon, breast, lung, liver, ovary, kidney and cervical carcinomas Soluble protein in gastrointestinal and prostate cancers, lymphomas and neuroblastoma
ULBP (human)	ULBP1, 2, 3 mRNA in heart, brain, lung, liver, thymus, testis, lymph nodes, tonsils and bone marrow ULBP4 mRNA mainly in skin and small intestine	Both mRNA and surface protein in melanoma, various leukaemias and carcinomas (stomach, colon, cervical and ovarian carcinomas for ULBP2 and ULBP3); ULBP1 and ULBP2 in T-cell lymphomas
Rae1 (mouse)	mRNA in embryonic tissues mRNA absent (or very rare) in healthy adult tissues	mRNA and surface protein in lymphomas, lung, prostate and cutaneous carcinomas (variable)
H60 (BALB/c mouse)	mRNA in embryonic tissues, adult thymus and spleen, but generally absent in healthy adult tissues	Both mRNA and surface protein in lymphomas, prostate and cutaneous carcinomas (variable)
Mult1 (mouse)	mRNA in thymus, spleen, lymph nodes, liver, gut, heart and lung	mRNA in T-cell lymphomas and sarcomas

*'Augmented' refers to transcription induction or increased surface protein expression, relative to 'basal' levels present in healthy tissues (Raulet, 2003, and references therein).
H60, histocompatibility antigen 60; MICA/B, MHC class I chain-related peptide A/B; MULT1, murine UL16-binding protein-like transcript 1; Rae1, retinoic acid early inducible gene 1; ULBP, UL16-binding protein.

presented by CD1d remains to be characterized, α-galactosylceramide (α-GalCer), which is extracted from the marine sponge *Agelas mauritianus*, is the most potent stimulator of NKT cells known so far and has proven to be effective against a wide range of tumours. α-GalCer protects against the development of primary sarcomas induced chemically or owing to a lack of genetic tumour suppression (Hayakawa *et al*, 2003), as well as against metastasis of multiple tumour cell lines: B16 (melanoma), EL4 (thymoma), Colon-26 (colon adenocarcinoma), 3LL (lung carcinoma), RM-1 (prostate carcinoma) and DA3 (mammary carcinoma) (Swann *et al*, 2004). In the absence of CD1d—as in CD1d$^{-/-}$ mice—methylcholanthrene-induced sarcomas developed faster and at a higher frequency than in wild-type mice (Crowe *et al*, 2002). Similar results were obtained using Jα18$^{-/-}$ mice (Smyth *et al*, 2000; Crowe *et al*, 2002), which selectively lack type I NKT cells, suggesting that these invariant lymphocytes are the crucial subset responsible for tumour suppression. Not unexpectedly, the transfer of wild-type type I NKT cells into Jα18$^{-/-}$ mice resulted in protection from tumour development. Murine NKT lymphocytes have been recently characterized as strikingly heterogeneous in their response to tumours; type I NKT lymphocytes eliminate CD1d^{+} cells, whereas type II NKT actually suppress tumour immunity in several mouse tumour models (Terabe *et al*, 2005). It has been shown that downregulation of tumour immunity by NKT cells might be accomplished through an IL-13 and TGF-β-dependent mechanism that shuts down anti-tumour CD8^{+} T lymphocytes (Terabe *et al*, 2003). Another group has also reported differential anti-tumour effects by subdividing NKT cells according to their tissue origin—thymus or liver—and CD4 expression (Crowe *et al*, 2005). Future research will therefore attempt to manipulate the balance between distinct NKT cell subsets towards the promotion of anti-tumour immunity.

Several studies of cancer patients suffering from a wide range of tumours have shown decreased numbers of NKT cells in the peripheral blood, as compared with healthy volunteers (Swann *et al*, 2004). Furthermore, deficient NKT-cell production of IFN-γ has been associated with the progression from pre-malignant to malignant multiple myeloma, and the NKT dysfunction could be corrected *in vitro* by culturing the NKT cells with α-GalCer-pulsed dendritic cells (Dhodapkar *et al*, 2003). After the disappointing outcome of the first clinical trial with soluble α-GalCer in patients with solid tumours (Giaccone *et al*, 2002), the use of α-GalCer-loaded dendritic cells has produced promising pre-clinical data in mice (Fujii *et al*, 2002) and in phase I human clinical trials (Nieda *et al*, 2004; Ishikawa *et al*, 2005); phase II trials are currently under way.

NKG2D ligands directly activate anti-tumour lymphocytes

The immunoreceptor NKG2D provides important stimulatory signals to NK and T cells (Bauer *et al*, 1999). In humans, NKG2D is constitutively expressed on the cell surface of NK, CD8^{+} T and γδ T lymphocytes, but is absent from CD4^{+} T cells and monocytes (Raulet, 2003). Several ligands for both the human and mouse NKG2D have been identified and, surprisingly, there is low amino-acid sequence homology between them. Human NKG2D binds to MHC I chain-related (MIC) peptides A and B (MICA and MICB), and to UL16-binding proteins (ULBP, members 1–4). Mouse NKG2D binds to retinoic acid early inducible gene 1 (Rae1), histocompatibility antigen 60 (H60), and murine UL16-binding protein-like transcript 1 (MULT1). MICA/B, H60 and MULT1 are transmembrane proteins, whereas ULBP1–4 and Rae1 localize to the cell surface using glycosylphosphatidylinositol (GPI) linkages. None of the NKG2D ligands bind to additional—peptide or lipid—antigens but rather interact directly with the receptor. In addition, in contrast to the MHC Ib molecules described above, NKG2D ligands do not associate with β2-microglobulin (Raulet, 2003). For some authors, the unorthodoxy of NKG2D ligands prompts their formal designation as 'MHC class I-related' instead of MHC Ib (Sullivan *et al*, 2006).

The murine ligands Rae1 and H60 are rare in healthy adult tissues, but their transcription is strongly induced in keratinocytes after their *in vivo* exposure to carcinogens (Girardi *et al*, 2001), and they are overexpressed in the cutaneous papillomas and carcinomas that subsequently develop, as well as in other tumours (Table 1). The expression of Rae1 or H60 by target cells was shown

to enhance cytolysis and the production of IFN-γ by anti-tumour CTLs (Diefenbach *et al*, 2000) and γδ T lymphocytes (Girardi *et al*, 2001), leading to tumour rejection *in vivo*. Furthermore, transfection of *rae1* or *h60* or *mult1* into NK-cell-resistant target cells made them susceptible to NK-cell-mediated killing and stimulated IFN-γ secretion by NK cells (Carayannopoulos *et al*, 2002; Cerwenka *et al*, 2000; Diefenbach *et al*, 2001). However, recent data suggest that IFN-γ can paradoxically downregulate H60 expression in tumours (Bui *et al*, 2006). Future experiments should try to reconcile these findings and elucidate the dynamics of H60 during tumour development *in vivo*.

The human MICA and MICB proteins—which are 91% identical at the amino-acid level—show a restricted and low expression in healthy tissues, but are strongly induced by cellular stress (including heat shock) and transformation, and accumulate in various tumour cell lines, particularly those of epithelial origin (Table 1). Atypically for MHC Ib molecules, the *MIC* genes are highly polymorphic: there are 61 *MICA* and 30 *MICB* alleles (Raulet, 2003). Although MICA/B was suggested to bind not only to NKG2D, but also to the Vγ1$^+$ TCR expressed by human intraepithelial γδ T lymphocytes (Wu *et al*, 2002), this claim is highly controversial. In fact, the identity of the majority of γδ TCR ligands remains an unresolved biological issue in which MHC Ib proteins are expected to have a crucial role (Steele *et al*, 2000; Sullivan *et al*, 2006; Thedrez *et al*, 2007). The membrane-bound form of MICA provides stimulatory signals to killer lymphocytes, whereas a soluble version (sMICA), shed from the cell surface by matrix metalloproteinases, systemically downregulates surface NKG2D and impairs tumour cytolysis mediated by T and NK cells, therefore constituting an important immune evasion mechanism for tumours (Groh *et al*, 2002; Salih *et al*, 2002).

Distantly related to the MIC proteins are the members of the ULBP family (23–26% amino-acid identity). In contrast to Rae1 or MICA, ULBPs are expressed at significant levels in a wide range of healthy tissues and cell lines of both epithelial and nonepithelial origin (Table 1; Cosman *et al*, 2001). Ectopic expression of ULBP1 or ULBP2 on murine EL4 or RMA tumour cells elicits potent anti-tumour responses in syngeneic B6 and SCID mice, recruiting NK, NKT and T cells to the tumour (Sutherland *et al*, 2006). Similarly, tumour cells that are insensitive to NK cells can be lysed effectively when transfected with ULBPs (Kubin *et al*, 2001).

Conclusion

In summary, NKG2D recognition of target tumour cells constitutes a potent anti-tumour mechanism, but its clinical application depends largely on the control and manipulation of the expression of NKG2D ligands. Therefore, the mechanisms controlling their specific regulatory signals need to be elucidated.

The immune system 'sculpts' tumours by selecting those that, owing to their reduced immunogenicity, escape its recognition/destruction. This process of elimination versus escape creates a dynamic relationship that has been termed 'cancer immunoediting' (Dunn *et al*, 2002). Non-classical MHC proteins can function as indicators of cellular transformation to anti-tumour lymphocytes, either directly or through the presentation of endogenous antigens that are (over-)expressed in tumours. Therefore, silencing MHC Ib expression might be a major immune evasion mechanism used by tumours, as described for Qa-2 during melanoma progression (Chiang *et al*, 2003). Another immune evasion strategy that involves MHC Ib proteins consists on the generation of soluble protein versions that block or internalize the corresponding receptors on lymphocytes, as mentioned for MICA (Groh *et al*, 2002; Salih *et al*, 2002). Similarly, the sustained surface expression of murine Rae1 has also been shown to have an inhibitory effect on NKG2D-expressing anti-tumour lymphocytes (Ogasawara *et al*, 2003; Oppenheim *et al*, 2005). Furthermore, CD1d-restricted type II NKT cells downregulate anti-tumour immunity (Terabe *et al*, 2005), whereas type I NKT cells promote tumour elimination. Such behaviour highlights the importance of determining the specific signals delivered by MHC Ib proteins to manipulate them towards protection. We believe that a better understanding of the underlying regulatory mechanisms of MHC Ib will make it possible to engineer vaccination approaches against various cancers that take advantage of the oligomorphism of most MHC Ib molecules.

ACKNOWLEDGEMENTS

We apologize to the authors whose work could not be cited owing to space constraints. We thank Fundação para a Ciência e Tecnologia of the Portuguese Ministry of Science and Technology [PTDC/SAU-MII/71662/2006] for support. B.S.-S. is a European Molecular Biology Organization (EMBO) Installation Grantee and acknowledges the EMBO's Young Investigator Programme.

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