Reviews

Non-immune Functions of MHC Class I Glycoproteins in Normal and Malignant Cells

(MHC / tumour / signal transduction)

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Abstract. MHC class I glycoproteins play a pivotal role in the regulation of immune responses by presenting antigenic peptides to cytotoxic T lymphocytes and by regulating cytolytic activities of natural killer cells. Cells originating in malignant tumours are often characterized by a profound immune escape phenotype. This phenotype is frequently associated with alterations in MHC class I-related antigen processing and presentation that enable tumours to escape immune surveillance. However, it now becomes clear that MHC class I molecules do not only provide a mechanistic framework for the presentation of antigenic peptides but, rather, possess broader biological functions due to their ability to regulate cell-to-cell communication and receptor-mediated trans-membrane signal transduction. In the present review we made an attempt to re-evaluate the significance of an altered MHC class I phenotype for tumour progression in view of the current state of knowledge concerning the aforementioned non-immune functions performed by these membrane glycoproteins.

The major histocompatibility complex (MHC) comprises a large cluster of genes region located on chromosome 6 in humans and on chromosome 17 in mice. This is a cluster of highly polymorphic genes, including those coding for class I and class II glycoproteins, as well as for many other proteins. Classical MHC class I alleles include HLA-A, HLA-B and HLA-C alleles in humans paralleled by H-2K, H-2D alleles in mice (H-2L region-encoded murine molecules are very similar but not identical to those encoded by H-2D) (Pamer and Cresswell, 1998). All class I molecules are composed of two separate immunoglobulin-like polypeptide chains: a membrane-integrated polymorphic heavy chain, which is non-covalently attached through its extracellular portion to a light chain (β2 microglobulin). The latter glycoprotein is encoded by a gene that does not reside in the MHC cluster (Pamer and Cresswell, 1998). A tight association of both subunits is of prime importance in stabilizing interactions between the peptide-binding cleft created by α1 and α2 domains of the heavy chain and antigenic peptides generated from endogenous proteins by specific proteolytic enzyme complexes, designated as a proteasome (Yewdell et al., 2003). The maturation of this three-party complex, i.e. heavy chain, β2 microglobulin and antigenic peptide, is an extremely complicated issue, which is beyond the scope of our review (Cresswell et al., 1999; Solheim, 1999).

Antigenic peptides residing in a class I peptide-binding cleft are recognized by a corresponding T-cell receptor (TCR) expressed at the cell surface of $CD8^+$ T lymphocytes. Interactions between a peptide-loaded class I molecule with the TCR and CD8 triggers a cascade of events that lead to T-cell proliferation, production of cytokines and lysis of the antigen-bearing target cell (Ramachandra et al., 1999; Seliger et al., 2000). In addition, MHC class I molecules regulate the lytic activities of natural killer (NK) cells, which possess killer immunoglobulin-like and C-type lectin superfamily receptors. These receptors are characterized by differential specificity and their engagement transduces either an activating or inhibitory signal to appropriate immunocytes expressing these receptors (Moretta et al., 1996; Lopez-Botet et al., 2000). Thus, MHC class I glycoproteins are mandatory for the regulation of immune responses directed against virally infected cells or cells altered by malignant transformation, since a deranged MHC class I expression ultimately alters both T and NK cell-mediated immunity. Therefore, it is not sur-

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Abbreviations: FGFR – fibroblast growth factor receptor, HLA – human leukocyte antigens, IGFR – insulin-like growth factor receptor, IL-2R – interleukin 2 receptor, IR – insulin receptor, MHC – major histocompatibility complex, PKB – protein kinase B, TCR – T-cell receptor.

prising that a deficiency in or an altered ratio between distinct MHC class I allele-encoded products, which is characteristic to a variety of malignancies, is of obvious relevance for tumour progression and the development of a metastatic disease (Segal et al., 1980; Isakov et al., 1984; Katzav et. al, 1985; Wallich et al., 1985; Hammerling et al., 1986; Eyal et al., 1990; Levin et al., 1991, 1992, 1994). For example, in the early 1980s we had demonstrated the existence of a clear correlation between the down-regulated expression of H-2K class I glycoproteins by certain murine tumour cells and their augmented metastatic capacities (Segal et al., 1980; Isakov et al., 1984). Correcting the H-2K status in these cells was found to abrogate their malignant and metastatic capacities (Wallich et al., 1985). The existence of a non-random selective pressure directed against the expression of certain types of MHC class I glycoproteins during tumour progression was substantiated by our study, where fifty percent of chemically induced murine fibrosarcomas were deficient for H-2K glycoproteins (Hammerling et al., 1986). Numerous additional studies also provided experimental evidence for this immune-escape strategy utilized by malignant cells (rev. by Garcia-Lora et al., 2003a).

MHC class I glycoproteins regulate transmembrane signal transduction and cell-to-cell communication

In addition to their well-known role in presentation of antigenic peptides to cytotoxic T cells, MHC class I glycoproteins could affect the cellular phenotype in a number of ways that are apparently unrelated to the immune response. Recent screening for genes regulating the development of precise inter-neuronal connections in the central nervous system revealed that genetically engineered mice that were unable to properly express MHC class I molecules exhibited abnormal connections between certain groups of neurons (Corriveau et al., 1998; Huh et al., 2000). A similar phenotype was also obtained in animals deficient for the expression of a determinative subunit of the TCR complex, i.e. CD3ζ. These data clearly indicated that MHC class I molecules may provide stimuli essential for brain development, stimuli, which are transduced through CD3ζ-associated receptors. Despite the obvious importance of these data, the nature of stimuli provided by MHC class I glycoproteins, as well as the nature of neuronal MHC receptor remain to be defined (Syken and Shatz, 2003).

The concept of possible non-immunological activities attributed to MHC class I glycoproteins is not novel and was raised in the late 1970s by us, as well as by other research groups, when class I-related molecules were detected in biological species lacking a conventional T cell-based adoptive immune system. Experiments employing polyclonal sera raised against mammalian MHC class I components provided evidence that plasma membranes of cells of *Drosofila melanogaster* and some other invertebrates contain antigenic determinants shared with β2 microglobulin and certain H-2 murine MHC class I molecules (Shalev et al., 1981, 1983; Scofield et al., 1982). In a related study, we have demonstrated that sera raised against β2 microglobulin induced mitogenic responses in lymphoid cells derived from *Cyprinus carpio* fish (Shalev et al., 1984). This finding, which implicated MHC molecules in the reception of mitogenic stimuli and transmembrane signal transduction, corresponded to previous data obtained by Meruelo and Edidin (1975). These authors reported that H-2 murine MHC class I molecules of distinct haplotypes differentially affect the intracellular concentration of a second messenger, i.e. cyclic adenosine monophosphate in liver cells. Changes in cellular behaviour produced by ligation of MHC class I molecules by specific antibodies were also demonstrated in other studies. The precise outcome of an anti-MHC antibody-induced signal depends on the cell type, additive, synergistic or antagonistic cross-talks with other MHC-unrelated signal generating cascades and, even, on the growth phase of the culture. For example, the research group led by Claesson reported that antibody-mediated cross-linking of HLA class I molecules at the cell surface of stationary human lymphoid cells activated ZAP70, $p56^{lck}$ (Skov et al., 1997a), Tyk2 and phosphatidylinositol 3 kinases (Skov et al., 1998). These changes were followed by a downstream cascade of biochemical events, which included activation of phospholipase Cγ (Skov et al., 1995), Jun N-terminus kinase (Skov et al., 1997b), increase in cytosolic Ca^{++} (Ruhwald et al., 1999), modulation of the activity of STAT3 transcription factors (Skov et al., 1998) and apoptotic cell death (Skov et al., 1997b). In contrast, exponentially growing cells reacted to antibody-induced stimuli by increasing their resistance to apoptotic cell death, presumably due to up-regulation of the Bcl-2 protein (Lamberth and Claesson, 2001). The described dualistic growth phase-dependent MHC signalling in lymphoid cells may have broad homeostatic consequences for the immune system. Namely, cell death could be prevented in activated proliferating and target cell-attacking effector lymphocytes or, alternatively, induced in anergic or senescent lymphocytes. In any case, the critical event in MHC-mediated signalling (either pro- or antiapoptotic) was the activation of membrane-associated *src*-related non-receptor tyrosine kinases ($p56^{lck}$), as was confirmed by exposing antibody-treated cells to inhibitors of tyrosine kinases (Skov et al., 1997a) or by performing the experiments on $p56^{lck}$-deficient cells (Lamberth and Claesson, 2001). The modulation of non-receptor-associated tyrosine kinases by MHC class I was also reported in human vascular endothelial and smooth muscle cells (Bian et al., 1997; Harris et al., 1997; Bian et al., 1998;

Bian and Reed, 1999; Nath et al., 1999). Ligation of anti-HLA-A, -B and -C antibodies to their epitopes expressed by vascular cells activated *src*-related and focal adhesion kinases. The activity of these kinases led to actin-dependent translocation of the fibroblast growth factor (FGF)-receptor from cytoplasmic compartments to the plasma membrane and nucleus and augmented proliferative responses of vascular cells to FGF. The authors suggested that host-derived antibodies directed against HLA epitopes expressed by vascular cells of grafted organs induced proliferation of the latter cells, thereby occluding blood vessels and leading to graft rejection.

Although the precise mode by which class I glycoproteins activate non-receptor tyrosine kinases was not defined in the aforementioned studies, the recruitment of second messengers following the ligation of an appropriate anti-HLA class I antibody to its cognate epitope implied that MHC class I glycoproteins function as or affect the function of membrane receptors. Since it has been clearly shown that the intracellular domain of the class I heavy chain is not essential or sufficient for signalling (Gur et al., 1999), it was unlikely that MHC class I molecules transduce a signal in a mode which is independent of other membrane molecules. Rather, it has been proposed that these membrane glycoproteins utilize their extracellular domains to complex with different membrane receptors. Indeed, many researches were able to demonstrate interactions of MHC class I molecules with receptors for peptide growth factors, such as insulin (IR), insulin-like growth factor (IGFR), epidermal growth factor, interleukin 2 (IL-2R), etc. (Stagsted, 1998). For example, there were numerous reports on co-precipitation of the IR with anti-MHC class I antibodies or *vice versa* (Chvatchko et al., 1983; Fehlmann et al., 1985; Phillips et al., 1986; Kittur et al., 1987; Ramalingam et al., 1997). Inter-molecular associations between the IR and MHC glycoproteins were also confirmed by other techniques, such as chemical cross-linking of [^{125} I]-labelled insulin to MHC class I complex (Verland et al., 1989) and fluorescence resonance energy transfer-based proximity measurements (Liegler et al., 1990; Ramalingam et al., 1997). A structural model proposed for these interactions implied that juxta-membrane constituents of the IR or the IGFR extracellular α chain displaces β2 microglobulin and associates with the α1 domain of the MHC class I heavy chain (Due et al., 1986; Stagsted et al., 1990; Olsson et al., 1994; Naranda et al., 1997). It has also been found that heavy chains belonging to some but not other haplotypes were able to form stable complexes with the IR (Reiland and Edidin, 1993). Associations between MHC and IR at the plasma membrane affected cellular capacity to bind insulin (Simonsen et al., 1985; Kittur et al., 1987; Ramalingam et al., 1997). For example, capping of MHC molecules with the appropriate antibodies was shown to inhibit insulin binding by murine leukaemia cells (Simonsen et al., 1985). Some authors also attributed a higher affinity of insulin binding to certain MHC haplotypes (Kittur et al., 1987; Reiland and Edidin, 1993). It has been reported that MHC : IR associations affected ligand-induced internalization of the receptor to intracellular compartments. Inhibition of MHC : IR interactions by exogenous peptides derived from either the α1 domain of murine H-2D class I or the receptor α chain increased the steady-state number of the cell-surface receptors and, presumably, made cognate ligands more potent (Due et al., 1986; Olsson et al., 1994; Naranda et al., 1997). Similar data were obtained for the IGFR as well (Naranda et al., 1997). The ability of the IR to complex with MHC molecules was also important for the ligand-induced post-receptor signalling cascade. For instance, it has been shown that in addition to augmenting the affinity of the receptor, the increased ratio of HLA-A heavy chain to IR expressed at the plasma membrane enhanced ligand-induced receptor autophosphorylation and recruited phosphatidylinositol 3-kinase (Ramalingam et al., 1997). The demonstration that these events could be inhibited by an excess of externally added β2 microglobulin corresponded to the aforementioned structural model for MHC : IR interactions (Due et al., 1986).

The nature of MHC: receptors interactions and their functional consequences could be better understood in view of the "membrane rafts" concept (Simons and Ikonen, 1997). This concept states that sphingomyelin- and cholesterol-enriched membrane micro-domains (rafts) accommodate a selected set of glycosylphosphatidylinositol-linked and other surface proteins, thereby leading to clustering and non-random distribution of different membrane molecules (Bartosz et al., 1981; Nagy et al., 2001, 2002). Amongst the proteins transiently or constantly residing in membrane microdomains are those tightly associated with signal transduction and cell-to-cell communication, such as non-receptor tyrosine kinases belonging to the Src family (Ilangumaran et al., 1999), some small and heterotrimeric GTPases (Prior and Hancock, 2001; Waheed and Jones, 2002), MHC class I and II (Szollosi et al., 1996), TCR/CD3 complex (Damjanovich et al., 1998), IR (Vainio et al., 2002), IL-2R (Damjanovich et al., 1998), ICAM-1 and -2 (Bene et al., 1994), cytoskeleton-regulating proteins (Caroni, 2001), etc. Lipid rafts are dynamic structures and their composition can be regulated by extracellular stimuli. Thus, insulin-induced translocation of the GLUT-4 transporter to the plasma membrane of adipocytes was shown to be mediated by the association of phosphorylated Cbl with lipid rafts, where it recruits the GTP-exchanging factor for Tc10 GTPase, followed by the re-arrangement of the actin cytoskeleton (Chiang et al., 2001; Maffucci et al., 2003). Vainio et al. (2002) have demonstrated that hepatocytes rapidly accumulate the

IR in lipid rafts when treated by insulin; this event and post-receptor signalling were precluded by exposing cells to a cholesterol-depleting agent. Being highly ordered structures with sometimes a non-uniform and asymmetrical distribution of their protein content, lipid rafts represent ideal platforms for precise reception and trans-membrane transduction of the extracellular signal (Damjanovich et al., 1998; Drake and Braciale, 2001). The modification or depletion of one raft component by any means (binding of ligand/antibody, chemical changes, mutation, etc.) would ultimately alter the concerted functioning of the other components. Matko et al. (2002) demonstrated that chemical disruption of raft-dependent clustering between IL-2 receptor chains, CD48, GM1 gangliosides and HLA glycoproteins abrogates IL-2-induced signals in T cells. These authors also suggested that HLA glycoproteins may stabilize rafts, presumably by dynamic associations with the cytoskeletal matrix Geppert and Lipsky, 1991; Kusumi and Sako, 1996).

Deregulated expression of MHC class I glycoproteins by malignant cells accentuates their malignant phenotype by non-immune mechanisms

The results of studies cited in the previous section strongly suggest that MHC class I glycoproteins are involved in the reception of extra-cellular signals and cell-to-cell communication; a process which is significantly impaired during malignant transformation and tumour progression. As aforementioned, the expression of class I molecules is impaired in many tumours. Therefore, a principal question arises, namely whether a deranged MHC expression by a given tumour cell can accentuate its malignant phenotype by disrupting proper receptor-mediated signal transduction. The relevance of non-immune functions of MHC class I to the biology of tumour cells was substantiated about fifteen years ago by independent studies performed by three different research groups. The experiments performed by each of these groups employed a model based on different B16 murine melanoma cell variants, i.e. B16-F1 and -F10 (Calorini et al., 1992), B78H1 (Lauri et al., 1993) and BL6 cells (Gorelik et al., 1993). All these cell variants were highly malignant, produced experimental metastases in syngenic mice and were deficient for the expression of H-2K murine MHC class I glycoproteins. Gene transfer-mediated reconstitution of H-2K (but not H-2D or H-2L) expression in these cells significantly attenuated their metastatic capacities not only when grafted to immunocompetent, as was previously shown by Katzav et al. (1985), but also to *immune compromised* mice (Calorini et al., 1992; Gorelik et al., 1993). These results led to the conclusion that certain types of MHC class I molecules could modulate the malignant potential of tumour cells also by mechanisms that are independent of proper presentation of antigenic peptides to effector immunocytes. When studying the possible non-immunological effects of MHC glycoproteins on the tumour cell phenotype, it has been found that the transfection of an H-2K-encoding gene significantly impairs the adherence capacities of transfectants (Calorini et al., 1992; Gorelik et al., 1993; Lauri et al., 1993). The impaired capacity of H-2K-transfected melanoma cells to bind cultured vascular endothelial cells and certain artificial matrices were likely mediated by a diminished expression of certain intergrin subunits (Lauri et al., 1993; Xu et al., 1998). Moreover, Gorelik et al. (1993) have shown that the *de novo* expression of H-2K glycoproteins in BL6-8 cells produced a dramatic change in the overall glycosylation pattern of membrane proteins, as manifested by an enhanced capacity of transfectants to bind certain lectins. Additional investigations revealed that these changes were associated with the up-regulation of α1,3-galactosyl transferase following the transfection of an H-2K-encoding gene into melanoma cells (Gorelik et al., 1995). The latter enzyme, which is attributed to the *trans*-Golgi compartment, competes with sialyl-transferases and diminishes sialylation of membrane molecules; Gorelik et al. (1995, 1997) provided evidence that the activity of sialyl-transferases was associated with the metastatic potential of BL6 and some other murine melanomas. In addition to their effects on glycosylation, H-2K class I molecules modulated other features of melanoma cells, such as the sensitivity to the cytotoxic effects of tumour necrosis factor α- and NK cell-mediated cytotoxicity (Kim et al., 1994) and the capacity of these tumour cells to induce apoptosis in co-cultured lymphoid cells (Fishman et al., 2001). These phenotypic alterations implied a profound change in the gene expression pattern associated with the reconstituted expression of an H-2K gene. Indeed, H-2K-expressing cells were characterized by the augmented expression of the tissue inhibitor of metalloproteinases 1 (Xu et al., 1998; Huang et al., 2000), an increased production of nitric oxide (Huang et al., 2000), an inhibited pigment synthesis (Prezioso et al., 1995) and a diminished production of the endogenous retrovirus (Li et al., 1996). It appeared that the "master" change produced by the *de novo* expression of the H-2K gene was the modulation of glycosylation processes, since H-2K-deficient cells transfected with the α1,3-galactosyl transferase-encoding gene adopted a phenotype similar to that developed by H-2K gene-transfected cells (Gorelik et al., 1993).

When considering the entire history of MHC signalling, including our own early observations (Shalev et al., 1983, 1984), we suggested that H-2K class I glycoproteins could alter the phenotype of melanoma cells by affecting the function and signalling pattern of membrane receptors for growth factors. Indeed, the stable expression of an H-2K-encoding gene in BL6

melanoma cells significantly augments their capacity to bind insulin due to an enhanced translocation of the IR to the plasma membrane (Assa-Kunik et al., 2003). The augmented insulin-binding capacity of H-2K-expressing cells correlated well with cell surface rather than intracellular compartmentalization of the IR in H-2K-expressing cells. In contrast, in H-2K-deficient cells most of the receptor molecules were retained in intracellular compartments and, therefore, were less available for binding of the externally added ligand. Accumulation of the IR in intracellular stores in the latter cells was not the result of an augmented internalization (Assa-Kunik et al., unpublished observations), but, rather, was associated with its glycosylation pattern, which was distinct from that detected in H-2K-expressing cells (Assa-Kunik et al., 2003). Noteworthy, the compartmentalization and glycosylation pattern of IGF-R in BL6 cells were also affected by H-2K glycoproteins in a way similar to that of the IR (Assa-Kunik et al, unpublished observations). These findings coincided with the aforementioned effects of H-2K glycoproteins on glycosylation enzymes in BL6 cells (Gorelik et al., 1993; Kim et al., 1993; Gorelik et al., 1995, 1997, 2001). The IR is known to be heavily glycosylated and an inappropriately glycosylated receptor cannot mature properly and be efficiently translocated to the plasma membrane (Hwang and Frost, 1999; Hwang et al., 2000). The retention of growth factor receptors in intracellular compartments due to deranged glycosylation was not unique to H-2K-deficient BL6 melanoma cells. Cytoplasmic accumulation of underglycosylated IGFR in oestrogen-independent breast carcinoma cells was previously reported by Dricu et al. (1999). The intracellular receptor was active and provided pro-survival stimuli, which reduced the cellular dependence on IGF. These authors, however, did not examine the relevance of MHC molecules for the observed phenomenon that they described. All H-2K-deficient (as compared to H-2K-expressing) BL6 melanoma cells, where most of receptor molecules were not expressed at the cell surface, were also characterized by a diminished dependence on insulin, IGF and some other growth factors (Assa-Kunik et al., 2003; Assa-Kunik et al., unpublished observations). Regardless whether the deficiency for H-2K glycoproteins and intracellular accumulation of the receptors were related phenomena or not, these findings indicated that the expression of certain MHC class I molecules influenced the transformed phenotype by tuning the cellular demand for growth factors. Apoptotic changes in H-2K-expressing cells maintained in growth factor-deprived (serum-free) culture medium were precluded by the addition of insulin, which rapidly activated protein kinase B (PKB/Akt). In contrast, the resistance of H-2K-deficient cells to growth factor starvation-induced apoptosis was strongly associated with a constitutive high activity of PKB/Akt detected in these cells (Assa-Kunik et al., 2003). We assume that yet unidentified ligands existing in the cytoplasm of H-2K-deficient cells could ligate intracellular IR, which, in turn, activates PKB/Akt. Another possible explanation for the ligand-independent high PKB/Akt activity was that the unique composition of lipid rafts in H-2K-deficient cells keeps PKB/Akt in a constitutively active form (Elhyany and Fishman, unpublished observations). The latter assumption corresponds to the aforementioned suggestion that MHC molecules may serve as a raft-stabilizing factor. The relationship between the IR, H-2K, PKB/Akt and the integrity of membrane rafts in BL6 melanoma cells is currently under intensive investigation in our laboratory.

In view of the above, one may assume that MHC class I glycoproteins may have evolved from a group of molecules which played a more general role in the regulation of membrane receptor-mediated signal transduction and cell-to-cell interactions in metazoan forms of life even before the occurrence of the adoptive lymphocyte-based immune system. One should not rule out the possibility that the ligation of MHC class I glycoproteins by specific receptors (either TCR, NK or other receptors) may regulate differentiation of normal cells as a fine tuning control mechanism of homeostasis and embryonic development. Therefore, imbalances in the expression of these molecules in tumour cells may play a cardinal role in determining the cellular malignant phenotype and not only the cellular ability to escape the immune surveillance.

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