Editorial

Prospects for Immunotherapy of MHC Class I-Deficient Tumours

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Cancer usually develops by a multistep process, characterized by the accumulation of genetic events within the tumour cell. These events are frequently associated with the activation of genes that are not normally expressed in somatic cells, with loss of function of the genes that are normally expressed in somatic cells and with mutations within these genes. The representatives of the newly activated genes have been designated as genes coding for tumour-associated antigens (TAA); the most important genes belonging to the deleted, downregulated or silenced genes are those encoding major histocompatibility complex (MHC) antigens. It has been demonstrated repeatedly that a high proportion (approximately 40–90%) of tumours derived from MHC class I^+ precursors are MHC class I deficient (for a review, see Garrido et al., 1997; Seliger et al., 1998; Tait, 2000; Campoli et al., 2002).

Several types of MHC class I downregulation have been described, including total, locus, allele and haplotype loss (for a review, see Algarra et al., 1997; Hicklin et al., 1999; Khong and Restifo, 2002). The MHC class I downregulation has serious consequences with regard to the immune reaction directed against TAAs. The TAA signal for activation of tumour-reactive lymphocytes is given by a short TAA peptide presented on the surface of tumour cells by MHC molecules. Partial or complete losses of the MHC class I molecules on tumour cells lead to the inability of the tumour cells to directly activate cytotoxic $CD8^+$ T lymphocytes (CTL), the major effector mechanism of the tumour defence reaction. Due to the redundancy of antigen cross-priming and direct priming of CTLs (Huang et al., 1994), even MHC class I^- tumour cells can induce T-cell immunity. However, the MHC class I^- tumour cells will not bind CTLs and will not be destroyed by circulating CTLs, so that due to the MHC class I restriction of the $CD8^+$ T cell-mediated immunity there is a higher probability that such tumour cells will escape from the immune surveillance (Levitsky et al., 1994). Indeed, decreased or absent MHC class I expression is frequently associated with the invasive and metastatic tumour phenotype.

The tumour defence mechanisms are mediated by both, MHC-restricted (T) cells and MHC-unrestricted (NK) cells. A high level of MHC class I molecule expression on tumour cells favours T cells as tumour defence effectors whereas the low MHC class I level favours NK cells. Therefore, the microheterogeneity of MHC class I expression in tumour cell populations, the balance of MHC-restricted and MHC-unrestricted defences as well as the immune selective pressure of CTL-mediated, NK cell-mediated, and other effector mechanisms decide the final outcome of the MHC class I expression on the primary tumour and its metastases as well as the final outcome of the tumour defence reaction.

In the majority of clinical trials it has been found that complete and partial responses to immunotherapeutic treatment modalities were rather rare. The relative paucity of responses could, at least in part, be due to the high proportion of MHC class I^- tumours in which the MHC class I downregulation was responsible for the resistance of the tumours to the major defence component, $CD8^+$ CTLs (Restifo et al., 1996; Jäger et al., 1997).

As compared to clinical trials, more optimistic results are being reported in experimental tumour systems. Mice with transplanted syngeneic tumours were successfully immunized against MHC class I^- melanoma, human papilloma virus (HPV) type 16-associated carcinoma and colon adenocarcinoma (Levitsky et al., 1994; Bubeník et al., 1999; Imboden et al., 2001). Tumour-inhibitory effects directed against MHC class I^- melanoma, HPV16-associated carcinoma and glioma were also observed after cytokine therapy (Nanni et al., 1998; Indrová et al., 2001, 2002, 2003; Mikyšková et al., 2001, 2003; Ehtesham et al., 2002). IL-12-transduced,

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Abbreviations: CTLs – cytotoxic T lymphocytes, GM-CSF – granulocyte-macrophage colony-stimulating factor, HPV – human papilloma virus, IFN – interferon, LAK – lymphokine-activated killer cells, LMP – low-molecular-weight polypeptide, MECL-1 – multicatalytic endopeptidase complex like-1, PA 28 – proteasome activator 28, MHC – major histocompatibility complex, NK – natural killer cells, TAA – tumour-associated antigen, TAP – transporter associated with antigen processing, TNF – tumour necrosis factor.

MHC class I$^-$ and MHC class II$^-$ B78H1 melanoma cells were used as a therapeutic vaccine in mice bearing micrometastases by non-transduced parental B78H1 cells. A substantial (80–90%) reduction in the number of lung metastases was obtained (Nanni et al., 1998). In an experimental model of HPV16-associated, metastasizing, MHC class I$^-$ and MHC class II$^-$ MK16 tumour (Šmahel et al., 2001) transplanted in syngeneic mice it has been demonstrated that peritumoral administration of IL-2 and IL-12 can inhibit growth of s.c. tumour transplants and reduce the number of their lung metastases. Similarly, IL-2 administration at the site of the MK16 tumour residua after surgery substantially reduced the percentage of tumour recurrences and the number of lung metastases (Mikyšková et al., 2001; Bubeník et al., 2003). Peritumoral administration of IL-2 gene-modified MK16 tumour vaccine also inhibited growth of s.c. MK16 tumour transplants and reduced the number of their lung metastases (Indrová et al., 2002; Bubeník et al., 2003). As tumour vaccines, either homologous tumour cells carrying an inserted IL-2 gene (MK16-IL-2) or unrelated tumour cells (Mc12-IL-2) which did not share any tumour rejection antigen with the MK16 cells and served exclusively as a local source of IL-2 production could be utilized for the therapy of the surgical minimal residual MK16 tumour disease (Bubeník et al., 1999, 2003).

Various mechanisms were reported to operate in these systems, such as NK cells (Levitsky et al., 1994; Imboden et al., 2001), NK-mediated antibody-dependent cellular cytotoxicity (Imboden et al., 2001), LAK cells (Falk et al., 2002) and CD4$^+$ T cells (Levitsky et al., 1994). Both, direct and indirect effects of the cytokines were apparently involved in the therapeutic activity. IL-12 could display its direct antiangiogenic properties, stimulation of IFNγ production by NK cells and stimulation of TNFα production (Nanni et al., 1998). IL-2 could enhance IFNγ production by both T and NK cells and in this way upregulate the expression of MHC class I and II molecules. Dendritic cells from MHC class I$^-$ tumour host bone marrow could activate CD8$^+$ and CD4$^+$ T cells by cross-priming. One might envision that the activated CD8$^+$ and CD4$^+$ T cells could produce IFNγ, which is capable of repairing some defects in the MHC class I pathway, and upregulate MHC class I expression on the tumour cell surface (Mikyšková et al., 2001; 2003; Indrová et al., 2002). The upregulation of the MHC class I expression could then induce sensitivity of the tumour cells to MHC class I-restricted immunity. Likewise, direct interaction of the MHC class I$^-$ tumour cells with NK cells could activate IFNγ production by the NK cells and upregulate the MHC class I expression and sensitivity to CTLs of the MHC class I$^-$ tumours.

MHC class I loss or downregulation in tumour cells can be due to the defects in MHC genes, due to the defects in the antigen processing and transport pathway (TAP-1 and -2, proteasome multicatalytic complex subunit LMP-2 and -7), or due to the alterations in binding of MHC class I transcription factor and regulation of MHC class I expression by methylation. The defects of TAP-1 and -2 as well as the defects of LMP-2 and -7 can be reversed by treatment with IFNγ or TNFα (reviewed in Hicklin et al., 1999; Seliger et al., 2000). IFNγ upregulates the expression of TAP-1 and -2 at the transcriptional level. Transfection of the TAP-1 gene into cells with deficient TAP-1 expression has been demonstrated to repair the TAP-1 production (Seliger et al., 1998; Kallfelz et al., 1999). In addition, IFNγ can also upregulate the expression of other components of the antigen-processing machinery, MECL-1 and PA28 (Fruh et al., 1999). Hence IFNγ has a potency to restore the pivotal components of the antigen-processing machinery and enhance the proteasome capacity to produce tumour peptides binding to MHC class I molecules and stimulating tumour rejection reactions (Skoskiewicz et al., 1985; Balkwill et al., 1987; Alimonti et al., 2000; Fromm and Ehrlich, 2001; Yoon et al., 2001; Ehtesham et al., 2002; Vilček, 2003; Garbi et al., 2003).

In the HPV16-associated tumour systems it has been shown that spleen cells from the MHC class I$^-$ tumour-immunized mice were not cytolytic when allowed to react *in vitro* with the MHC class I$^-$ target cells. However, when the MHC class I$^-$ cells were cultivated *in vitro* in the presence of IFNγ, they acquired, together with the expression of MHC class I molecules, the sensitivity to the MHC class I-restricted cytolytic effect of the spleen cells from the MHC class I$^-$ tumour-immunized mice (Indrová et al., 2002).

In general, the MHC class I status of most tumours treated in immunotherapeutical experiments is poorly defined or unknown, both in the experimental model systems (Bubeník, 2002) and in clinical trials. The prospects of immunotherapy of the MHC class I$^-$ tumours are critically dependent on the character of the MHC class I defect in the respective tumour. If the MHC class I defect is reparable with IFNγ, various therapeutic protocols based on the stimulation of endogenous IFNγ production or administration of exogenous IFNγ may be utilized (Tatake et al., 1993; Boehm et al., 1997; Kaplan et al., 1998). In addition to the MHC class I upregulating effects, IFNγ has also the advantage of directly inhibiting tumour growth and angiogenesis (Kakuta et al., 2002).

Another prospective approach to the development of therapeutic protocols aiming at upregulation of MHC class I molecule expression prior to or simultaneously with the immunotherapy is demethylation of the silenced MHC gene promoters. Treatment of human breast carcinoma cells with histone deacetylase inhibitors and 5-aza-2'-deoxycytidine was shown to display direct antineoplastic activity (Primeau et al., 2003). Demethylation of melanoma cell lines upregulated the expression of MAGE-1 tumour antigen

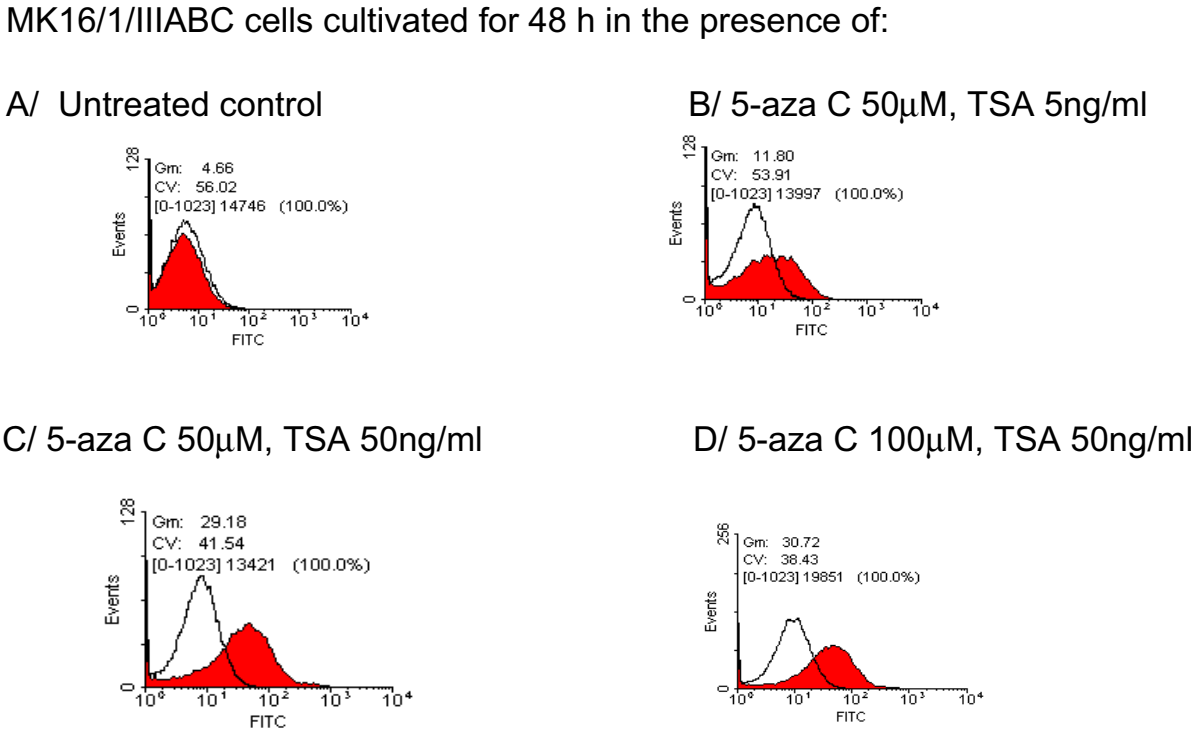

Fig. 1. Induction of MHC class I expression on MK16/1/IIIABC tumour cells by demethylation (5-aza-2' -deoxycytidine, 5-aza-C) and acetylation (histone deacetylase inhibitor, trichostatin A, TSA) agents. Detection of MHC class I molecules by flow cytometry with ELITE flow cytometer (Coulter, Miami, FL) and FITC-anti-mouse H-2 K^b/H-2D^b antibody (clone 28-8-6, Pharmingen, CA). (Hejnar, J., Reiniš, M., and Bubeník, J., unpublished results).

(Weber et al., 1994; deSmet et al., 1996); likewise, downregulation of HLA-G molecules could be reversed by demethylation (Moreau et al., 2003). Treatment with 5-aza-2'-deoxycytidine was also successful as a therapy of patients with high-risk myelodysplastic syndrome (Wijermans et al., 2000). In the MHC class I- tumour systems it has been found that MHC class I expression can be upregulated not only by cultivation of the tumour cells in the presence of IFNγ, but also by cultivation in the presence of 5-aza-2'-deoxycytidine and histone deacetylase inhibitor, trichostatin A (Fig. 1). Demethylation and acetylation agents certainly belong to the prospects of immunotherapy in MHC class I- tumour systems.

In conclusion, more attention should be paid to the MHC class I status of the neoplasms during the elaboration of tumour immunotherapy protocols. It may be envisaged that the immunotherapeutic protocols will differ in tumours expressing MHC class I molecules and in MHC class I-deficient tumours. If the tumour is MHC class I-deficient, it is important to learn whether the respective MHC class I deficiency can be *in vitro* repaired with IFNγ (defects of TAP-1 and -2, LMP-2 and -7, MECL-1, PA28) or with TNFα (Lu et al., 2001). In such tumour systems the cytokine therapy with exogenous IFNγ, IL-12, IL-2, GM-CSF and TNFα, or therapy with tumour vaccines carrying an inserted cytokine gene and constitutively producing IFNγ, IL-12, IL-2, GM-CSF or TNFα should be considered. Prospectively, the therapeutic role of the induction of MHC class I expression by demethylation and acetylation agents prior to or simultaneously with the immunotherapy should also be investigated.

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