Short Communications

The Role of NK1.1$^+$ Cells in the Protection against MHC Class I$^+$ HPV16-Associated Tumours

(HPV16 / MHC class I expression / NK1.1$^+$ cells)

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Abstract. Depletion of NK1.1$^+$ cells by repeated i.p. injections of PK136 antibody significantly enhanced growth of MHC class I$^+$ tumours in syngeneic mice. Depletion starting before tumour transplantation or on the day of transplantation substantially accelerated tumour growth; depletion starting on day 7 or 14 after tumour transplantation was without any effect. These results indicate that the NK1.1$^+$ cells play an important inhibitory role during the early phase of the growth of some MHC class I$^+$ tumours. Since the relevant target for NK cells is a „missing self" signal, absence of the MHC class I molecules, the NK cells cannot be expected to directly inhibit the growth of the MHC class I$^+$ tumours. The results indicate that the effects of non-NK cells or indirect effects mediated by NK cell interactions and release of cytokines were responsible for the results.

In a series of pilot experiments we have found that the depletion of NK1.1$^+$ cells significantly enhanced growth not only of MHC class I deficient, but also MHC class I proficient tumours. These unexpected results indicated that some MHC class I$^+$ tumours can be inhibited by NK1.1$^+$ cells despite the absence of the conventional NK cell targets on the surface of the MHC class I$^+$ cells, the „missing self" signal (Karre et al. 1986; Ljunggren and Karre, 1990).

Material and Methods

Mice

C57BL/6 males, 2–4 months old, were obtained from AnLab Co., Prague, Czech Republic.

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Abbreviations: HPV – human papilloma virus, MHC – major histocompatibility complex, NK – natural killer.

Tumour cell lines

The MHC class I$^+$ TC-1 tumour cell line was established by transformation of primary C57BL/6 mouse lung cultures with HPV16 *E6/E7* oncogenes and activated *Ha-ras* (Lin et al., 1996). The MHC class I deficient TC-1 cell subline TC-1/A9 (a generous gift from Dr. M. Šmahel) was derived from TC-1 tumours formed in mice preimmunized repeatedly with HPV16 *E7*-containing plasmid DNA (Šmahel et al., 2003). The expression of MHC class I molecules on tumour cells was determined by cytofluorometric analysis with FITC-anti-mouse H-2K^b/H-2D^b monoclonal antibody (clone 28-8-6, Pharmingen, San Diego, CA). As an isotype control, FITC-labelled antibody of irrelevant specificity (clone 155-178, Pharmingen) was used. As can be seen in Fig. 1, TC-1 tumour cells are MHC class I$^+$ and TC-1/A9 tumour cells are MHC class I deficient.

In vivo cell depletion and FACS analysis

To study the role of NK1.1$^+$ cells involved in the early phase of the growth of HPV16-associated murine tumours, *in vivo* depletion of NK1.1$^+$ was performed using monoclonal antibody PK136 (Koo et al., 1986). To deplete the effector cells, 0.1 mg of the antibody was i.p. injected into mice, in the first week 3 times a week and in the following two weeks once a week. Tumour cells were transplanted s.c. in the dose of 1×10^4 TC-1 cells or 5×10^3 TC-1/A9.

Depletion of cells was monitored in spleens on days 0 and 7 by flow cytometry using monoclonal antibody PE-anti mouse NK1.1 (clone PK136, Pharmingen). As an isotype control, PE-labelled antibody of irrelevant specificity (clone G155-178, Pharmingen) was used.

Statistical analyses

For statistical analyses of differences between the growth curves of tumours, the analysis of variance from NCSS, Number Cruncher Statistical System (Kaysville, UT), statistical package was used.

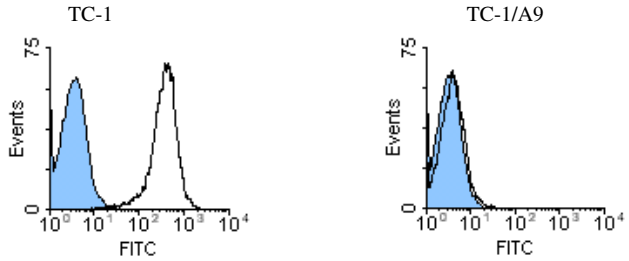

Fig. 1. Flow cytometric analysis of MHC class I expression on TC-1 and TC-1/A9 cells. Tumour cells were stained with FITC-anti-H-2K^b/H-2D^d monoclonal antibody (open histograms) or with isotype control antibody (filled histograms).

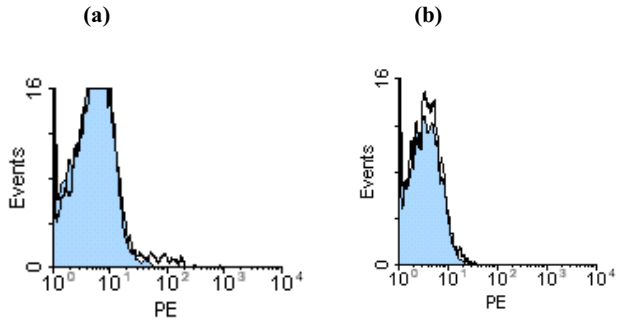

Fig. 2. Presence of NK1.1$^+$ cells in spleens of C57BL/6 mice (a) prior to depletion and (b) after depletion of NK1.1$^+$ cells. Cells were stained with PE-anti-mouse NK1.1 monoclonal antibody (open histograms) or with isotype control antibody (filled histograms).

Results and Discussion

NK1.1$^+$ cell depletion starting before transplantation of MHC class I$^+$ tumours

Mice were depleted of NK1.1$^+$ cells by repeated injections of 0.1 mg of PK136 antibody on days 8, 6, 3, and 0 before and on days 7 and 14 after transplantation of the TC-1 tumour cells or on days 0, 2, 5, 7, 14 and 21 after transplantation of the TC-1 cells. Depletion of the NK1.1$^+$ cells was monitored by flow cytometry and the results shown in Fig. 2 have confirmed that the depletion was complete.

Depletion of the NK1.1$^+$ cells starting before transplantation or on the day of transplantation substantially accelerated tumour growth of MHC class I$^+$ TC-1 tumours (Fig. 3a, 3b). To compare the effect of NK1.1$^+$ cell depletion on the growth of the MHC class I$^+$ and MHC class I$^-$ tumours, MHC class I deficient subline of the TC-1 cells, the cell line TC-1/A9 was utilized. As expected, depletion of the NK1.1$^+$ cells substantially enhanced growth of the MHC class I deficient TC-1/A9 tumours (Fig. 3c).

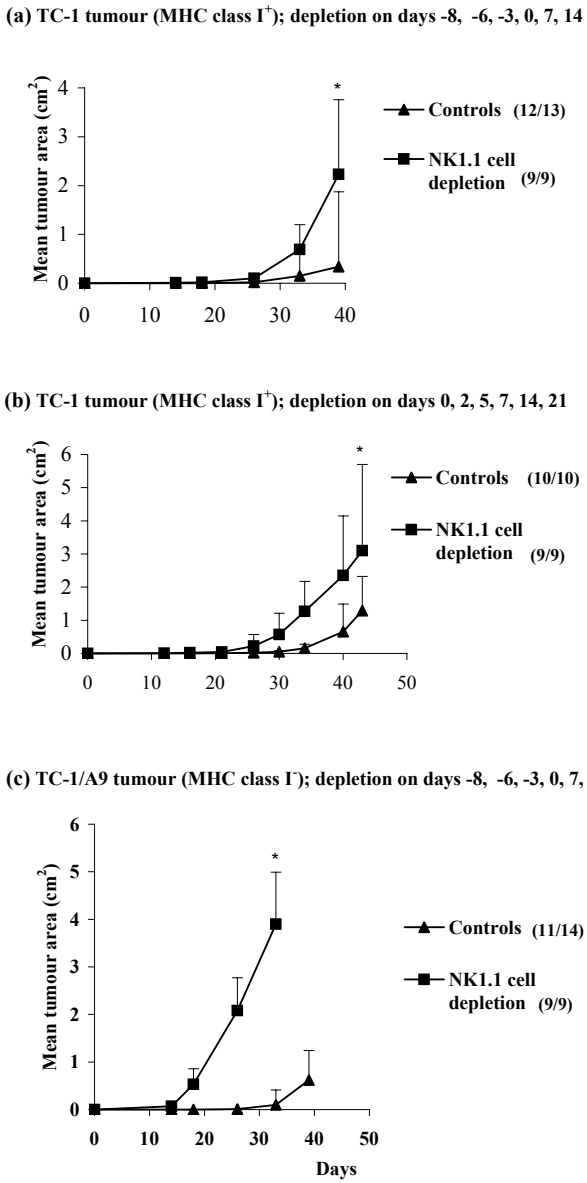

Fig. 3. NK1.1$^+$ cell depletion starting before transplantation of tumour cells (No. of tumour-bearing mice/Total No. of mice).
*P < 0.01 as compared to the controls.

NK1.1$^+$ cell depletion starting after transplantation of MHC class I$^+$ tumours

Mice were depleted of NK1.1$^+$ cells by repeated injections of 0.1 mg of PK136 antibody on days 7, 9, 12, 14, 21 and 28 or on days 14, 16, 19, 21 and 28 after transplantation of TC-1 cells. Depletion of NK1.1$^+$ cells performed according to this experimental schedule was without any effect on the tumour growth (Fig. 4). The results have shown that the strong enhancing effect of NK1.1$^+$ cell depletion on the growth of MHC class I proficient TC-1 tumours was time-dependent. Whereas NK1.1$^+$ cell depletion starting before transplantation or on the day of transplantation of tumours accelerated

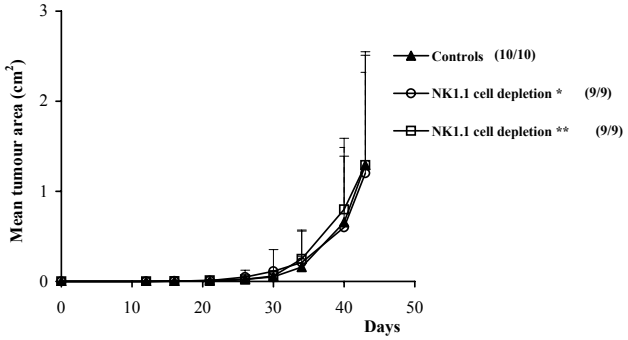

Fig. 4. NK1.1$^+$ cell depletion starting after transplantation of TC-1 (MHC class I$^+$) tumour cells (No. of tumour-bearing mice/Total No. of mice)
*Depletion on days 7, 9, 12, 14, 21, 28.
**Depletion on days 14, 16, 19, 21, 28.

tumour growth, depletion starting after transplantation of tumour cells was without any effect.

These results indicate that the NK1.1$^+$ cells play an important inhibitory role during the early phase of the growth of some MHC class I$^+$ tumours. Since the relevant target for NK cells is a „missing self" signal, the absence of the MHC class I molecules (Karre et al., 1986; Ljunggren and Karre, 1990), according to the papers published in the late eighties the NK cells could not be expected to directly inhibit the growth of the MHC class I$^+$ (TC-1) tumours. Consequently, the results would indicate that the effects of non-NK cells or indirect effects mediated by NK cell interactions and release of cytokines were responsible for the results. However, expression of some ligands for NK cells on the tumour cell surface can allow them to participate in the anti-tumour response. It has later been reported that NK cells were able to reject tumours expressing MHC class I molecules if the tumours expressed a RAE-1 ligand for NKG2D molecules (Cerwenka et al., 2001). Similarly, the role of NKT cells in the anti-tumour response should also be considered. In the C57BL6 mice, which we have used, the NK1.1 molecules are expressed not only on the NK, but also on the NKT cells (Shimizu et al. 2004). The immunostimulating activity of the NKT cells depends on the cytokine/co-stimulatory milieu (Smyth et al., 2002). It has been demonstrated that the early growth inhibition of MHC class I$^+$ tumours required the transfer of not only CD8$^+$, but also NKT cells from immunized animals (Stewart et al. 2003).

Experiments are in progress to establish whether NKG2D molecules, the effect of NKT cells or other mechanisms are responsible for the unexpected results in our tumour system.

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