

**Prof. Gabriele Multhoff** PhD, Department of Hematology and Oncology, Molecular Oncology, University of Regensburg

Prof. Dr. Gabriele Multhoff is heading the research group “NK cells in immunotherapy”. For 10 years Prof. Multhoff has had a research interest in the development of NK cell mediated immunotherapeutic approaches based on heat shock proteins (HSP). Together with Dr. Claus Botzler, Prof. Multhoff founded the biotechnology company multimmune GmbH in 1999. Prof. Multhoff’s research group aims to identify a tumour-specific membrane expression of Hsp70 that correlates with an increased sensitivity to lysis mediated by NK cells *in vitro* and *in vivo*.

#### Research Interest within the TRANSNET project:

Hsp70-activated NK cells mediating GvL reactivity

Screening of bone marrow of ALL patients by multiparameter flow cytometry revealed that leukemic blasts but not normal bone marrow cells do present Hsp70 on their plasma membrane. Therefore, we speculated that membrane-bound Hsp70 might serve as a leukemia-specific target structure for the stimulation of a leukemia-specific immune response. Furthermore, we could demonstrate *in vitro* that the anti-leukemic effect of NK cells, but not of T cells, could be further enhanced by preincubation of NK cells with an immunostimulatory Hsp70-peptide. Previous results indicated that a 14-mer peptide of the major stress inducible Hsp70 is able to stimulate proliferation, cytolytic activity and migration of NK cells towards Hsp70 membrane positive leukemic blasts.

The principle of the immunostimulatory effects of Hsp70 peptide against Hsp70-positive tumors is illustrated in Figure 1.

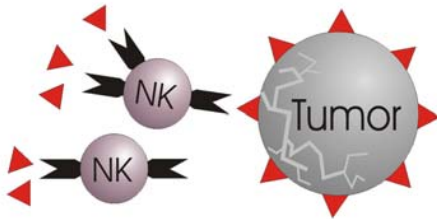
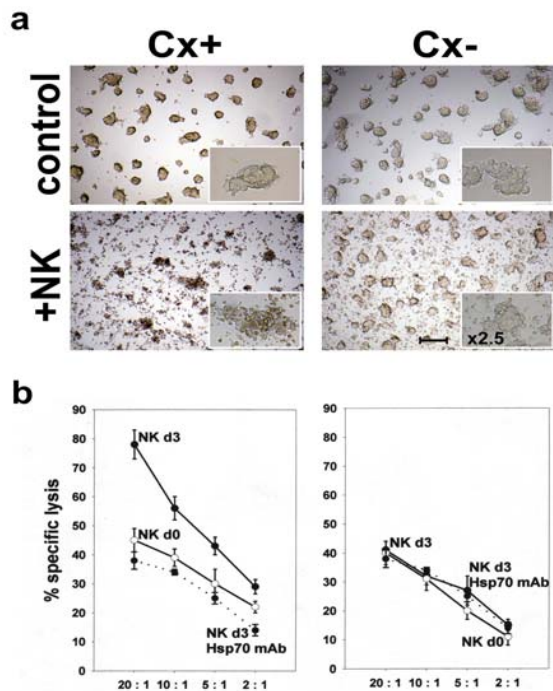


Figure 1: Hsp70 peptide marked as red triangles stimulate NK cell activity

The Hsp70-activity of NK cells after stimulation with Hsp70-peptide was demonstrated by migration assays (Gastpar et al JI, 2003), standard Cr-51 release assays and Granzyme B ELISPOT technique. A representative example of the migratory capacity of NK cells selectively towards Hsp70 membrane-positive tumor target cells *in vitro* is illustrated in Figure 2. Allogeneic model tumor cells either carrying Hsp70 on their plasma membrane (CX+) or not (CX-) served as target cells. After co-incubation period with Hsp70-activated NK cells, it appeared that Hsp70 membrane-positive tumor cells attract Hsp70-activated NK cells, whereas Hsp70 membrane-negative tumor cells failed to do so. Furthermore, cell viability of CX+ tumor cells was drastically reduced as illustrated in the inset showing CX+ cells attacked by NK cells at a higher magnification. This finding could be confirmed in a cell mediated lympholysis assays, showing significantly enhanced cytolytic activity of Hsp70 membrane-positive tumor cells by Hsp70-activated NK cells (NK d3) as compared to unstimulated NK cells (NK d0). The final proof that membrane-bound Hsp70 serves as a the recognition structure for these NK cells was demonstrated by antibody blocking assays (dotted line in each graph).

Figure 2: a) Co-incubation assay of Hsp70 membrane positive (CX+) and –negative (CX-) tumor cells with *in vitro* Hsp70-activated NK cells (upper graph) and b) a comparison of the cytolytic function of unstimulated (NK d0) and Hsp70-peptide stimulated (NK d3) NK cells (lower graph). Hsp70 antibody blocking studies (dotted line) reveal that the enhanced cytolytic activity of NK cells against tumor target cells is mediated through Hsp70.



In addition to these findings, the cytolytic activity of NK cells could be correlated with the production and the release of granzyme B. Granzyme B could be identified as the key apoptosis-inducing agent for Hsp70 membrane-positive tumor cells (Gross et al, 2003). Membrane-bound Hsp70 was found to facilitate binding and uptake of granzyme B. Regarding these results, our laboratory established a granzyme B ELISPOT assays that provides a non-radioactive alternative to the Cr-51 release assay, for rapid determination of the activation status of Hsp70-reactive NK cells. Using these techniques the immunostimulatory capacity of allogeneic and autologous Hsp70-activated NK cells are currently tested against leukemic blasts with differential Hsp70 membrane expression pattern.

#### Selected Patents:

G.Multhoff, An Hsp70 peptide stimulating NK cell activity and uses thereof G.Multhoff, Novel use of Hsp70 protein

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