[Frontiers in Bioscience 13, 3448-3456, May 1, 2008]

# Soluble NKG2D ligands: prevalence, release, and functional impact

**Helmut Rainer Salih[1], Stefan Holdenrieder[2], Alexander Steinle[3]**

*[1]Department of Internal Medicine II, Eberhard-Karls-University, Tuebingen, Germany, [2]Institute of Clinical Chemistry, Ludwig-Maximilians-University, Munich, Germany, [3]Department of Immunology, Eberhard-Karls-University, Tuebingen, Germany*

## TABLE OF CONTENTS



## 1. ABSTRACT

Natural Killer (NK) cells are capable to recognize and eliminate malignant cells. Anti-tumor responses of NK cells are promoted by the tumor-associated expression of cell stress-inducible ligands of the activating NK receptor NKG2D. Current evidence suggests that established tumors subvert NKG2D-mediated tumor immunosurveillance by releasing NKG2D ligands (NKG2DL). Release of NKG2DL has been observed in a broad variety of human tumor entities and is thought to interfere with NKG2D-mediated tumor immunity in several ways. Further, levels of soluble NKG2DL (sNKG2DL) were also found to be elevated under various non-malignant conditions, although the functional implications remain largely unclear. Here we review and discuss the available data on the prevalence, release, functional impact, and potential clinical value of sNKG2DL.

## 2. INTRODUCTION

Since their discovery in 1975, Natural Killer (NK) cells are known for their ability to attack and kill tumor cells *in vitro* and to eliminate tumors *in vivo* (25, 26, 36, 76). NK cell recognition of tumor cells is guided by the principles of 'missing-self' and 'induced-self' which imply that cells with a low or absent expression of MHC class I ('missing-self') and a stress-induced expression of ligands of activating NK receptors ('induced-self') are preferentially recognized and eliminated by NK cells (3, 41, 55). NK cells sense MHC class I molecules by ITIM-bearing inhibitory NK receptors. Meanwhile, many inhibitory NK receptors including members of the human killer-immunoglobulin-like receptors (KIR), members of the mouse Ly49 receptors, or the CD94/NKG2A receptor, and their MHC class I ligands are well characterized (38). In contrast, 'positive' recognition of tumor cells by NK

cells is only partially understood, in particular, because tumor-associated ligands of major activating NK receptors have not yet been identified (38, 46). One notable exception and, with regard to the 'induced-self' recognition mode, paradigmatic example is the NKG2D receptor which interacts with a multitude of cell stress-induced, MHC class I-related molecules (12, 54).

## 3. THE NKG2D/NKG2DL-SYSTEM

### 3.1. The activating NKG2D receptor

NKG2D is an activating, homodimeric, type II transmembrane, C-type lectin-like receptor (CTLR) and, like many other immune-related CTLR, is encoded in the Natural Killer Gene Complex (NKC) on the short arm of human chromosome 12 (3, 58, 79). In humans, NKG2D is expressed on virtually all cytotoxic lymphocytes including NK cells, γδ T cells, and CD8 αβ T cells in association with the adaptor protein DAP10 (3, 77). Upon NKG2D engagement, DAP10 is phosphorylated and recruits both the p85 subunit of the phosphatidylinositol-3-kinase (PI3K) and a Grb2-Vav1 intermediate initiating NK cytotoxicity (69, 77). In mice, NKG2D is present on almost all NK cells, a subpopulation of γδ T cells and activated, but not naive CD8 T cells (54). There is also an alternative NKG2D splice form in mice (called NKG2D-S), but not in humans, which allows activated NK cells to recruit the ITAM-bearing DAP12 adaptor (54). Engagement of NKG2D by its MHC class I-related ligands results in the activation of effector mechanisms of NK cells and γδ T cells, and in the costimulation of CD8 αβ T cells (3, 20, 54). A pronounced expression of NKG2D ligands (NKG2DL) renders even MHC class I-positive tumor cells susceptible to NK cell cytotoxicity (3, 38).

### 3.2. Human NKG2DL

In humans, NKG2DL comprise two members of the MHC class I-related chain (MIC) family (MICA, MICB) and six members of the UL16-binding protein (ULBP) family of proteins (ULBP1-4, RAET1G, RAET1L) (2, 12, 17, 67). MICA and MICB molecules are encoded by tandem genes within the MHC adjacent to the HLA-B locus (2). The MICA locus is highly polymorphic with more than 60 alleles known to date (http://www.anthonynolan.org.uk/HIG/). MICA*08 is by far the most frequent allele and encodes for a MICA protein with a truncated cytoplasmic domain due to a frameshift mutation (2). MIC molecules are type I transmembrane glycoproteins consisting of three extracellular domains (α1, α2, α3), a hydrophobic membrane-spanning domain and a cytoplasmic domain. The α1 and α2 domains form an MHC class I-like fold followed by an immunoglobulin-like α3 domain much alike an MHC class I heavy chain (40). However, unlike classical MHC class I molecules, MIC molecules do not present antigenic peptides nor associate with β2-microglobulin (18). In healthy tissue, MICA protein expression appears to be restricted to epithelial cells of the gastrointestinal tract which was attributed to stimulation by the intestinal flora (18, 66).

ULBP are encoded by a gene cluster on the long arm of chromosome 6. Their MHC class I-like α1α2 superdomain is only distantly related to MIC molecules (~ 25% amino acid sequence identity), they lack an immunoglobulin-like α3 domain and some ULBP are GPI-anchored (2, 8, 12, 17, 67). Altogether, there are eight human NKG2DL known to date, raising questions about the evolutionary cause of this redundancy. There is emerging evidence that NKG2DL differ in their affinity for NKG2D, in their expression pattern, and are differentially targeted by viral immunoevasins (2, 12, 17, 67) suggesting that NKG2DL redundancy evolved to serve different immune functions and/or to counter viral immune escape strategies.

### 3.3. Mouse NKG2DL

All known mouse NKG2DL have an MHC class I-like ectodomain similar to ULBP (α1α2 platform domain, no α3 domain), but markedly differ at the sequence level, and comprise members of the GPI-anchored retinoic acid early inducible-1 (RAE1) family of proteins (RAE1α, -β, -γ, -δ, and -ε), the minor histocompatibility antigen H60, and the murine UL16-binding protein–like transcript 1 (MULT1) (38, 54). No MIC homologues have been found in mice. Crystallographic analyses revealed that the various NKG2DL, in spite of highly diversified primary sequences, form a similar tertiary structure which is engaged by NKG2D homodimers in a comparable mode of binding (39, 40, 52). Expression of RAE1 molecules, but not H60 or MULT1, has been shown to be inducible by ligation of Toll-like receptors (24). Further studies are needed to address differences in expression and/or function of mouse NKG2DL.

### 3.4. Stress-inducible expression of NKG2DL

Since surface expression of NKG2DL potentially marks cells for destruction by cytotoxic lymphocytes, it is evident that NKG2DL expression must be tightly regulated. Accordingly, the current conception is that NKG2DL usually are not expressed on "healthy" tissue, but rather are inducibly expressed upon cellular stress, e. g. accompanying viral infection or malignant transformation (15, 71). For example, NKG2DL expression is strongly up-regulated by cells infected with herpesviruses or tumor cells subjected to genotoxic stress (15, 42, 74). Genotoxic stress triggers the DNA damage response (DDR) which appears, at least in part, to account for the tumor-associated expression of NKG2DL, since NKG2DL expression on tumor cells was shown to be dependent on the DDR-mediator ATM (ataxia telangiectasia, mutated) (14). Accordingly, MICA and MICB are broadly expressed by malignant cell lines, epithelial tumors and leukemias which raised the question of an involvement of NKG2D in tumor immunosurveillance (21, 37, 60).

## 4. NKG2D AND TUMOR IMMUNOSURVEILLANCE

Several lines of evidence now point to a crucial role of NKG2D in tumor immunosurveillance: Firstly, *in vivo* studies demonstrated that ectopic expression of NKG2DL strongly stimulates anti-tumor immune responses in mice against otherwise tumor-forming malignant cell lines (7, 9). NKG2DL-expressing tumor cells are vigorously rejected by NK cells and/or $CD8^+$ T cells by

means of perforin-based cytotoxicity, whereas IFN-γ and TRAIL do not appear to be of major importance in this process (7, 9, 64). NKG2D dysfunction caused by persistent NKG2DL exposure resulted in the failure to reject NKG2DL-expressing tumor cells (49, 75). There is also evidence that rejection of NKG2DL-positive tumor cells leads to the generation of memory T cells which are protective against subsequent challenges with parental NKG2DL-negative tumor cells (9).

Secondly, MICA/B are broadly expressed by epithelial tumors, melanoma, neuroblastoma, and various hematopoietic malignancies and expression of mouse NKG2DL is up-regulated during tumorigenesis (16, 21, 34, 53, 60, 70). As described above, a recent study suggests that tumor-associated expression of MICA/B is intrinsically coupled to malignant processes via the DDR suggesting that NKG2DL act as 'immuno-alerters' for cancer cells (14).

Thirdly, in mouse models of spontaneous or chemically-induced tumors, NKG2D was shown to be protective against tumor development (49, 63).

Fourthly, in tumor samples of 449 patients with colorectal tumors, enhanced MICA expression significantly correlated with an improved disease-specific survival (73).

Collectively, these findings provide ample evidence that the NKG2D/NKG2DL system acts as an important tumor immunosurveillance mechanism allowing the recognition and elimination of malignant cells and may counteract the development of neoplasms already at an early stage. During cancerogenesis, however, anti-tumor immunity also results in selection of cancer cells capable of evading the immune response (11). Escape from NKG2D-mediated tumor immunosurveillance may be achieved by silencing NKG2D or abrogation/attenuation of NKG2DL expression. For example, TGFβ, which is frequently produced by tumors, appears to play an eminent role as it has been reported to down-regulate both MICA and NKG2D surface expression on tumors and NK cells, respectively (6, 13). As it turns out, the release of soluble NKG2DL by tumor cells is another major mechanism subverting the NKG2D/NKG2DL surveillance system.

## 5. SOLUBLE NKG2DL

### 5.1. Soluble NKG2DL in humans

In 2002, we and the group of Spies independently reported that MICA molecules are released from tumor cells in a soluble form (23, 62). Elevated levels of soluble MICA (sMICA) were found in sera of patients with various gastrointestinal malignancies, breast and lung tumors, melanoma, and leukemia (23, 60, 62). High levels of sMICA were also detected in transgenic mice constitutively and ubiquitously expressing MICA suggesting that release of sMICA is not an exclusive property of malignant cells (75). Considering the close sequence relationship of MICA and MICB, it did not come as a surprise that MICB is also released from tumor cells such as hepatoma cells and detectable in sera of patients with epithelial or hematopoetic malignancies (1, 43, 60, 61). Interestingly, therapeutic agents have been shown to affect the expression of MIC molecules and thereby also the levels of sMIC molecules: for example, treatment of hepatoma cells with the histone-deacetylase inhibitor valproate enhanced cell surface expression of MICA and MICB, but not of ULBP1-3, and this was accompanied by increased release of sMICA and soluble MICB (sMICB) (1).

Only few reports exist on soluble ULBP molecules: tumor cells have been shown to release soluble ULBP2 (sULBP2) *in vitro,* and elevated sULBP2 levels were detected in some hematopoetic malignancies, but not in gastrointestinal malignancies (48, 72). For the transmembranous ULBP molecules ULBP4/RAET1E and RAET1G, the existence of soluble forms as a result of alternative splicing has been postulated, but neither sULBP4 nor sRAET1G have yet been detected in human samples (5, 12). Since *in vivo* expression and function of these ULBP molecules are poorly defined, the biological relevance of sULBP4 and sRAET1G remains unclear. There are no reports on soluble mouse NKG2DL.

### 5.2. Mechanisms of NKG2DL release

In our initial study, we reported that a broad-range metalloprotease-inhibitor (MPI) reduced levels of sMICA detectable in supernatants of tumor cells and concomitantly caused accumulation of MICA on the tumor cell surface (62). The apparent molecular mass of tumor cell-derived sMICA protein is similar to the mass of the recombinant MICA ectodomain (62). Altogether, this suggests that MICA is shed from the surface of tumor cells by metalloproteases. Meanwhile, we provided evidence that a similar mechanism also is involved in the release of sMICB and sULBP2 (61, 72). These experiments also revealed that MICA shedding is strongly enhanced upon PMA-treatment pointing to the involvement of ADAM proteases (72). Gastric tumor cell lines treated with very high concentrations of an inhibitor of phosphatidylinositol-specific phospholipase C (PI-PLC) exhibited increased cell surface levels of ULBP1 and ULBP2 (65). Based on these findings, the authors speculate that PI-PLC may also be involved in shedding of ULBP molecules (65). Very recently, MICA was shown to be associated with endoplasmic reticulum protein 5 (Erp5) on the tumor cell surface with Erp5 function being strictly required for MICA shedding (35). Erp5 forms transitory, disulfide-linked complexes with MICA inducing a substantial conformational change in the α3 domain of MICA which promotes MICA shedding (35).

### 5.3. Prevalence of sMICA in malignant diseases

Up to now, particularly sMICA levels, but also other soluble NKG2DL (sNKG2DL) have been analyzed in a multitude of different malignancies. Table 1 provides an overview on presently available studies on sMICA and other sNKG2DL in sera of patients with malignant diseases. Elevated levels of sMICA were found in sera of patients with various cancers including gastrointestinal, hepatocellular, ovarian, pancreatic, prostate, and non-small cell lung carcinoma, melanoma, neuroblastoma, and thymoma (e.g. (10, 23, 28, 31, 34, 35, 43 ,53, 62, 78)).

**Table 1.** sNKG2DL in sera of patients with malignant diseases

| Maligancy | sNKG2DL | Observation | Reference |
|---|---|---|---|
| AML, ALL, CML | MICA, MICB | Elevated levels in patient sera | 60 |
| Breast, lung, melanoma | MICA | Elevated levels in patient sera | 23 |
| Various epithelial cancers | MICA | Elevated levels, correlation with cancer stage and metastasis | 28 |
| Various epithelial cancers | MICB | Elevated levels, correlation with cancer stage and metastasis | 29 |
| CML | MICA | Elevated levels in patient sera, reduction after Imatinib therapy | 4 |
| colorectal cancer | MICA/B | Elevated levels in patient sera, inverse correlation with NKG2D+ NK cells | 10 |
| HNSCC | MICA | No detectable levels in patient sera | 57 |
| Liver cancer | MICA | Elevated levels, association with cancer stage | 33 |
| Melanoma, NSCLC | MICA | Elevated levels in patient sera | 32 |
| Multiple myeloma | MICA | Elevated levels, correlation with paraprotein levels and survival | 56 |
| Neuroblastoma | MICA | Elevated levels in patient sera | 53 |
| NHL, AML, CML | ULBP2 | Elevated levels in hematopoietic maligancies, not detected in GIT | 72 |
| Pancreas | MICA, MICB | Elevated levels in patient sera | 43 |
| Prostate | MICA | Elevated levels, associated with cancer stage and NKG2D downregulation | 78 |
| Stomach | MICA | No difference between sera of patients and healthy controls | 50 |
| Stomach, colon, rectum | MICA | Elevated levels in patient sera | 62 |
| Stomach, colon, rectum | MICB | Elevated levels in patient sera | 61 |
| Thymoma | MICA | Elevated levels in patient sera | 31 |

Abbreviations: NSCLC, non-small cell lung cancer; HNSCC, head and neck squamous cell carcinoma; GIT, gastrointestinal tumors

However, elevated levels of sMICA were not only found in patients with epithelial and other solid tumors, but also in sera of patients with various hematopoietic malignancies such as acute myeloid and lymphoid leukemia, chronic myeloid leukemia (CML), and multiple myeloma (4, 56, 60). Two studies on patients with gastric cancer or head and neck carcinoma did not observe differences in sMICA levels in cancer patients as compared to healthy controls (50, 57). It remains unclear whether these negative results are due to technical issues or, for example, may reflect population-dependent (allelic) differences in MICA expression and release.

**5.4. Soluble MICA - a novel tumor marker?**

Owing to the broad tumor-associated expression and shedding of MIC molecules, determination of sMICA in patient sera may be expected to be valuable as an immunological tumor marker. Most studies performed to date found higher sMICA concentrations in individuals with cancer diseases, particularly in those with advanced stages of cancer, as compared to healthy controls. However, the small patient numbers investigated and the use of healthy blood donors as controls instead of individuals with organ-specific benign diseases, which are more relevant for differential diagnosis, limits the clinical value of these reports. Hence, we analyzed sMICA serum levels in a large cohort of patients. The over 500 samples comprised sera from individuals with various epithelial cancers (including colorectal and various other gastrointestinal cancers, lung cancer, breast cancer, ovarian and other gynecologic cancers, renal and prostate cancer), individuals with clinically relevant benign diseases as well as healthy individuals (28). Levels of sMICA in healthy donors were undetectable or in the low value range while pre-therapeutic serum levels in patients with various malignancies were significantly higher. However, various benign diseases were also associated with elevated serum levels of MICA molecules limiting the diagnostic capacity of sMICA for cancer disease. Particularly infectious diseases as well as hepatic and renal diseases potentially affecting the marker metabolism lead to considerably higher levels of sMICA molecules (27). However, sMICA levels distinguished significantly between benign and malignant diseases in general as well as in the subgroups of lung and gynecological cancers, and elevated sMICA levels correlated significantly with cancer stage and metastasis (28). A higher frequency of sMICA positivity in high grade versus low grade hepatocellular carcinoma (HCC) patients suggested that increase of sMICA was correlated with disease progression of HCC, while no elevated sMICA were detected in sera of chronic hepatitis patients or healthy individuals (33). Similarly, disease progression in patients with prostate cancer correlated with an increase of sMICA levels (78).

Due to the correlation of sMICA with cancer stage one might expect that sMICA will provide prognostic information. Rebmann and coworkers found a significant correlation between elevated sMICA levels and a poor overall as well as a poor progression-free survival in multivariate analyses in patients with multiple myeloma (56).

Soluble MICA may be of value not only as diagnostic or prognostic marker, but also for monitoring cancer growth in response to therapeutic interventions or treatments with agents known to affect NKG2DL expression such as imatinib mesylate and histone deacetylation inhibitors (1, 4). Detailed future studies will have to clarify the pros and cons of sMICA and other sNKG2DL as tumor markers.

**5.5. Soluble MICA versus soluble MICB**

Analysis of sMICB in sera of more than 500 individuals including 296 patients with diverse epithelial cancer and 154 patients with benign diseases (29) revealed that there were relatively few cancer patients with elevated sMICB levels as compared to sMICA, and thus, sMICB levels did not significantly differ from healthy donors (29). Soluble MICB also failed to discriminate effectively between cancers and the relevant organ-specific benign diseases due to the considerable number of individuals with ‘sMICB-negative’ cancer disease. In about 70% of cancer patients, there were no sMICB levels detectable while only

**Table 2.** sNKG2DL in sera of patients with non-malignant conditions

| Condition | sNKG2DL | Observation | Reference |
|---|---|---|---|
| Rheumatoid arthritis | MICA | Elevated levels in patient sera | 19 |
| Infections, renal insufficiency, cholestasis | MICA, MICB | Elevated levels in patient sera | 27 |
| Pregnancy | MICA/B | Elevated levels in sera of prenant women | 45 |
| Celiac disease | MICA | Elevated levels, inverse correlation with gluten-free diet | 30 |
| During follicular phase of cycle | MICA | Elevated levels, predictive of implantation failure and abortion | 51 |
| Heart transplantation | MICA | Elevated levels, inverse correlation with rejection | 68 |

about 20% of patients were 'sMICA-negatives'. The weak correlation found between sMICA and sMICB levels may potentially reflect a different functional role of the two molecules (61). While there is some evidence that MICB expression is more tightly controlled compared to MICA (18, 74) this needs to be addressed in greater detail. Despite the low correlation of sMICA and sMICB, the combined use of both markers did not yield an improved clinical sensitivity (29).

It is noteworthy that serum levels of both sMICA and sMICB correlated significantly with the extent of disease as defined by the classification of the *Union Internationale Contre le Cancer* (UICC): While there was no association between sMICA and sMICB levels and tumor size, cell differentiation (grading), or lymph node involvement, both markers showed a clear correlation with the presence of distant metastasis. Thus, one may speculate that the reduction of MICA/B surface expression by shedding and the effects of sMICA and sMICB in serum on host lymphocyte NKG2D expression and function (as discussed in detail in chapter 6) might play a role in late stages of tumor progression by overcoming the confining effect of NK cells and CD8 T cells. Up to now there are no comprehensive data on the value of sULBP molecules as markers in cancer disease.

### 5.6. Soluble MIC benign diseases

Cell-stress induced expression of NKG2DL is not restricted to malignancies. Thus it is not surprising that elevated levels of sNKG2DL have been observed in patients with various non-malignant diseases (see Table 2). First data of sNKG2DL, i.e. sMICA, in non-malignant diseases were provided by the group of Spies in a report on the expansion of $NKG2D^{+}CD4^{+}CD28^{-}$T cells associated with autoreactivity in patients with rheumatoid arthritis (19). In this study, elevated levels of sMICA presumably derived from MICA-expressing synoviocytes were detected in patient sera. In contrast, in the vast majority of sera of 141 patients with hepatic autoimmune processes sMICA and sMICB levels were not increased (27). Elevated sMICA levels were also found in sera of patients with celiac disease, while control sera from patients with non-coeliac disease enteropathy were sMICA-negative (30). Interestingly, significantly more patients under gluten-containing diet had elevated sMICA levels compared to patients with gluten-free diet (30).

A recent report implicated a role of sMICA in heart allograft rejection and graft outcome (68). About 60% of patients had elevated sMICA serum levels after heart transplantation, while no sMICA was detected prior to surgery. Interestingly, absence of sMICA after transplantation was significantly associated with severe rejection reactions, and in the majority of patients with good graft status, sMICA levels remained elevated.

It is noteworthy that sNKG2DL not only arise in pathological conditions, but also in physiological processes. For example, a study reported on elevated levels of sMICA/B in sera of pregnant women and sMIC release by placental explants *in vitro* (45). Another study observed that increased sMIC levels in infertile women during the follicular phase of the cycle preceding *in vitro* fertilization (IVF) were associated with implantation failure (51). Furthermore, in case of successful implantation, high sMIC levels were predictive of spontaneous abortion. This led the authors to consider sMICA as a marker to evaluate chances for successful pregnancies after IVF (51).

Taken together, release of sNKG2DL is not an exclusive phenomenon of malignancies, but also occurs under various physiological conditions and non-malignant pathophysiological situations. How sNKG2DL interfere with the immune system in these processes remains to be elucidated.

## 6. INTERFERENCE OF NKG2DL RELEASE WITH TUMOR SURVEILLANCE

There is increasing evidence that the release of sNKG2DL by tumor cells has a negative impact on NKG2D-mediated tumor surveillance.

Firstly, an obvious consequence of NKG2DL shedding by tumor cells is the reduction of cell surface NKG2DL density and, concomitantly, a reduced susceptibility for NKG2D-mediated cytotoxicity (62, 72). In fact, *in vivo* studies with NKG2DL-expressing tumor cells demonstrated that NKG2D-mediated anti-tumor responses are strongly dependent on NKG2DL surface levels, and thus diminishing NKG2DL surface levels by shedding is likely to impair anti-tumor responses (9).

Secondly, sMICA has been implicated in the systemic down-regulation of NKG2D on NK cells and CD8 T cells in cancer patients: sMICA in cancer patient sera was shown to downregulate NKG2D on CD8 T cells and NK cells *in vitro* and to be associated with a systemic reduction of NKG2D surface expression on cytotoxic lymphocytes *in vivo* (23). NKG2D-downregulation in patients where sMICA was undetectable was attributed to the failure to detect lower levels of sMICA or to other sNKG2DL (23). It was also shown that binding of sMICA to NKG2D leads to the internalization of the NKG2D-MIC complex, downregulation of surface NKG2D and in turn impairment of the responsiveness of tumor-antigen-specific effector T cells (23). NK cells of patients with colorectal or prostate

cancer also displayed reduced levels of NKG2D, and analysis of multiple samples from normal donors and patients revealed an inverse correlation between the NKG2D surface expression by NK cells and the serum levels of sMIC (10, 78). In patients with CML a substantial decrease of sMICA was observed after Imatinib therapy accompanied by a restored NKG2D expression on $CD8^+$ T cells and NK cells (4). Similarly, increased levels of sMICA in serum of patients with hepatocellular carcinoma (HCC) and neuroblastoma were associated with down-regulated NKG2D expression and impaired activation of NK cells (33, 53). In addition, sMICA derived from advanced HCC also impaired NK cell induced maturation and activation of DC (33). In other studies, NKG2D-downregulation by sNKG2DL was not observed indicating that this process may be more complex than suspected. In our studies, we did not observe downregulation of NKG2D on the NK cell line NKL, neither by sMICA nor by sMICB, nor by sULBP2 derived from culture supernatants of the respective C1R-transfectants (61, 72). In a study of gastric cancer patients, NKG2D expression on tumor-infiltrating $CD8^+$ T cells was remarkably reduced and recovered after surgical removal of the tumors. NKG2D downregulation required direct contact between gastric cancer cells and T cells, whereas soluble factors did not affect NKG2D expression (50). In experiments taking advantage of MICA-transgenic mice, NKG2D was down-regulated on non-transgenic NK cells after coculture with MICA-expressing splenocytes, but not after exposure to sMICA-containing mouse serum (75). NKG2D down-regulation as a consequence of direct contacts between NKG2D-bearing lymphocytes and NKG2DL-expressing target cells has also been reported in several other studies (e. g. (23, 24, 47, 49, 61, 72)). Mechanistically, NKG2D down-regulation in this setting is ascribed to NKG2DL-induced endocytosis of NKG2D as well as synaptic transfer of NKG2D to target cells resulting in an impairment of NKG2D-mediated cytotoxicity (23, 44, 59). In non-malignant diseases, NKG2D on intraepithelial lymphocytes (IEL) from patients with active celiac disease was not down-regulated by recombinant sMICA (30). In patients with celiac disease or rheumatoid arthritis (RA), NKG2D on T cells was not down-regulated *in vivo* despite the presence of high levels of sMICA in patient sera (19, 30). It was suggested that the negative effect of sMICA on NKG2D expression is counteracted by the presence of TNF and IL-15 in RA (19). A recent *in vitro* study utilizing genetically modified human T cells expressing a chimeric NKG2D receptor found that addition of recombinantly produced sMICA did not greatly affect NKG2D-mediated cytotoxicity even at concentrations ~100-fold exceeding sMICA levels in sera of tumor patients (80).

Thirdly, Spies and colleagues reported that sMICA drives the expansion of immunosuppressive $CD4^+NKG2D^+$ T cells in late-stage human tumor settings. These $CD4^+$ $NKG2D^+$ T cells suppress proliferation of $CD4^+NKG2D^+$ T cells via secretion of Fas ligand and thus may promote tumor immune escape (22).

Collectively, these data strongly support the notion that release of MIC molecules by tumor cells counteracts cancer immunosurveillance by NK and T cells. Consequently, one may suggest that a therapeutic blockade of MIC release or neutralisation of shed sMIC would support immunological approaches of treating cancer. Interestingly, in the course of previous immunotherapeutical regimens, an emergence of MICA-specific antibodies was observed which was accompanied by a reduction of circulating sMICA and an augmentation of NK and $CD8^+$ T cell cytotoxicity (32).

## 7. PERSPECTIVE

Current concepts of cancer immunosurveillance state that the immune system can take actions against arising tumors, but ultimately, tumors often evolve strategies to escape from immune control. An increasing body of evidence collected in the last five years suggests that release of sNKG2DL may aid the evasion of tumor cells from immunosurveillance by cytotoxic lymphocytes. Therefore it is not only of immediate scientific, but also of clinical interest to further explore molecular mechanisms and cellular functionalities associated with the release and impact of sNKG2DL. A thorough understanding of the underlying processes may pave the way to more efficacious immunological therapies against cancer.

## 8. ACKNOWLEDGMENTS

We apologise to all investigators whose studies were not cited in this article due to space constraints. The authors are supported by grants of the DFG, Deutsche Krebshilfe, and Wilhelm Sander-Stiftung.

## 9. REFERENCES

1. S. Armeanu, M. Bitzer, U. M. Lauer, S. Venturelli, A. Pathil, M. Krusch, S. Kaiser, J. Jobst, I. Smirnow, A. Wagner, A. Steinle and H. R. Salih: Natural killer cell-mediated lysis of hepatoma cells via specific induction of NKG2D ligands by the histone deacetylase inhibitor sodium valproate. *Cancer Res* 65,6321-6329 (2005)
2. S. Bahram, H. Inoko, T. Shiina and M. Radosavljevic: MIC and other NKG2D ligands: from none to too many. *Curr Opin Immunol* 17,505-509 (2005)
3. S. Bauer, V. Groh, J. Wu, A. Steinle, J. H. Phillips, L. L. Lanier and T. Spies: Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* 285, 727-729 (1999)
4. N. Boissel, D. Rea, V. Tieng, N. Dulphy, M. Brun, J. M. Cayuela, P. Rousselot, R. Tamouza, P. Le Bouteiller, F. X. Mahon, A. Steinle, D. Charron, H. Dombret and A. Toubert: BCR/ABL oncogene directly controls MHC class I chain-related molecule A expression in chronic myelogenous leukemia. *J Immunol* 176,5108-5116 (2006)
5. W. Cao, X. Xi, Z. Hao, W. Li, Y. Kong, L. Cui, C. Ma, D. Ba and W. He: RAET1E2, a soluble isoform of the UL16-binding protein RAET1E produced by tumor cells, inhibits NKG2D-mediated NK cytotoxicity. *J Biol Chem* 282, 18922-18928 (2007)
6. R. Castriconi, C. Cantoni, C. M. Della, M. Vitale, E. Marcenaro, R. Conte, R. Biassoni, C. Bottino, L. Moretta and A. Moretta: Transforming growth factor beta 1 inhibits

expression of NKp30 and NKG2D receptors: consequences for the NK-mediated killing of dendritic cells. *Proc Natl Acad Sci U S A* 100, 4120-4125 (2003)
7. A. Cerwenka, J. L. Baron and L. L. Lanier: Ectopic expression of retinoic acid early inducible-1 gene (RAE-1) permits natural killer cell-mediated rejection of a MHC class I-bearing tumor *in vivo*. *Proc Natl Acad Sci U S A* 98, 11521-11526 (2001)
8. D. Cosman, J. Mullberg, C. L. Sutherland, W. Chin, R. Armitage, W. Fanslow, M. Kubin and N. J. Chalupny: ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. *Immunity* 14, 123-133 (2001)
9. A. Diefenbach, E. R. Jensen, A. M. Jamieson and D. H. Raulet: Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. *Nature* 413, 165-171 (2001)
10. E. S. Doubrovina, M. M. Doubrovin, E. Vider, R. B. Sisson, R. J. O'Reilly, B. Dupont and Y. M. Vyas: Evasion from NK cell immunity by MHC class I chain-related molecules expressing colon adenocarcinoma. *J Immunol* 171,6891-6899 (2003)
11. G. P. Dunn, A. T. Bruce, H. Ikeda, L. J. Old and R. D. Schreiber: Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol* 3, 991-998 (2002)
12. R. A. Eagle and J. Trowsdale: Promiscuity and the single receptor: NKG2D. *Nat Rev Immunol* 7, 737-744 (2007)
13. G. Eisele, J. Wischhusen, M. Mittelbronn, R. Meyermann, I. Waldhauer, A. Steinle, M. Weller and M. A. Friese: TGF-beta and metalloproteinases differentially suppress NKG2D ligand surface expression on malignant glioma cells. *Brain* 129, 2416-2425 (2006)
14. S. Gasser, S. Orsulic, E. J. Brown and D. H. Raulet: The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. *Nature* 436, 1186-1190 (2005)
15. S. Gasser and D. H. Raulet: The DNA damage response arouses the immune system. *Cancer Res* 66, 3959-3962 (2006)
16. M. Girardi, D. E. Oppenheim, C. R. Steele, J. M. Lewis, E. Glusac, R. Filler, P. Hobby, B. Sutton, R. E. Tigelaar and A. C. Hayday: Regulation of cutaneous malignancy by gammadelta T cells. *Science* 294,605-609 (2001)
17. S. Gonzalez, V. Groh and T. Spies: Immunobiology of human NKG2D and its ligands. *Curr Top Microbiol Immunol* 298, 121-138 (2006)
18. V. Groh, S. Bahram, S. Bauer, A. Herman, M. Beauchamp and T. Spies: Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. *Proc Natl Acad Sci U S A* 93, 12445-12450 (1996)
19. V. Groh, A. Bruhl, H. El Gabalawy, J. L. Nelson and T. Spies: Stimulation of T cell autoreactivity by anomalous expression of NKG2D and its MIC ligands in rheumatoid arthritis. *Proc Natl Acad Sci U S A* 100, 9452-9457 (2003)
20. V. Groh, R. Rhinehart, J. Randolph-Habecker, M. S. Topp, S. R. Riddell and T. Spies: Costimulation of CD8alphabeta T cells by NKG2D via engagement by MIC induced on virus-infected cells. *Nat Immunol* 2, 255-260 (2001)
21. V. Groh, R. Rhinehart, H. Secrist, S. Bauer, K. H. Grabstein and T. Spies: Broad tumor-associated expression and recognition by tumor-derived gamma delta T cells of MICA and MICB. *Proc Natl Acad Sci U S A* 96,6879-6884 (1999)
22. V. Groh, K. Smythe, Z. Dai and T. Spies: Fas-ligand-mediated paracrine T cell regulation by the receptor NKG2D in tumor immunity. *Nat Immunol* 7, 755-762 (2006)
23. V. Groh, J. Wu, C. Yee and T. Spies: Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. *Nature* 419, 734-738 (2002)
24. J. A. Hamerman, K. Ogasawara and L. L. Lanier: Cutting edge: Toll-like receptor signaling in macrophages induces ligands for the NKG2D receptor. *J Immunol* 172, 2001-2005 (2004)
25. Y. Hayakawa and M. J. Smyth: Innate immune recognition and suppression of tumors. *Adv Cancer Res* 95, 293-322 (2006)
26. R. B. Herberman, M. E. Nunn and D. H. Lavrin: Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic acid allogeneic tumors. I. Distribution of reactivity and specificity. *Int J Cancer* 16, 216-229 (1975)
27. S. Holdenrieder, P. Eichhorn, U. Beuers, W. Samtleben, P. Stieber, D. Nagel, A. Peterfi, A. Steinle and H. R. Salih: Soluble NKG2D ligands in hepatic autoimmune diseases and in benign diseases involved in marker metabolism. *Anticancer Res* 27, 2041-2045 (2007)
28. S. Holdenrieder, P. Stieber, A. Peterfi, D. Nagel, A. Steinle and H. R. Salih: Soluble MICA in malignant diseases. *Int J Cancer* 118,684-687 (2006)
29. S. Holdenrieder, P. Stieber, A. Peterfi, D. Nagel, A. Steinle and H. R. Salih: Soluble MICB in malignant diseases: analysis of diagnostic significance and correlation with soluble MICA. *Cancer Immunol Immunother* 55, 1584-1589 (2006)
30. S. Hue, J. J. Mention, R. C. Monteiro, S. Zhang, C. Cellier, J. Schmitz, V. Verkarre, N. Fodil, S. Bahram, N. Cerf-Bensussan and S. Caillat-Zucman: A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease. *Immunity* 21, 367-377 (2004)
31. S. Hue, R. C. Monteiro, S. Berrih-Aknin and S. Caillat-Zucman: Potential role of NKG2D/MHC class I-related chain A interaction in intrathymic maturation of single-positive CD8 T cells. *J Immunol* 171, 1909-1917 (2003)
32. M. Jinushi, F. S. Hodi and G. Dranoff: Therapy-induced antibodies to MHC class I chain-related protein A antagonize immune suppression and stimulate antitumor cytotoxicity. *Proc Natl Acad Sci U S A* 103, 9190-9195 (2006)
33. M. Jinushi, T. Takehara, T. Tatsumi, N. Hiramatsu, R. Sakamori, S. Yamaguchi and N. Hayashi: Impairment of natural killer cell and dendritic cell functions by the soluble form of MHC class I-related chain A in advanced human hepatocellular carcinomas. *J Hepatol* 43, 1013-1020 (2005)
34. M. Jinushi, T. Takehara, T. Tatsumi, T. Kanto, V. Groh, T. Spies, R. Kimura, T. Miyagi, K. Mochizuki, Y. Sasaki and N. Hayashi: Expression and role of MICA and MICB in human hepatocellular carcinomas and their

regulation by retinoic acid. *Int J Cancer* 104, 354-361 (2003)
35. B. K. Kaiser, D. Yim, I. T. Chow, S. Gonzalez, Z. Dai, H. H. Mann, R. K. Strong, V. Groh and T. Spies: Disulphide-isomerase-enabled shedding of tumour-associated NKG2D ligands. *Nature* 447, 482-486 (2007)
36. R. Kiessling, E. Klein and H. Wigzell: "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. *Eur J Immunol* 5, 112-117 (1975)
37. L. L. Lanier: A renaissance for the tumor immunosurveillance hypothesis. *Nat Med* 7, 1178-1180 (2001)
38. L. L. Lanier: NK cell recognition. *Annu Rev Immunol* 23, 225-274 (2005)
39. P. Li, G. McDermott and R. K. Strong: Crystal structures of RAE-1beta and its complex with the activating immunoreceptor NKG2D. *Immunity* 16, 77-86 (2002)
40. P. Li, D. L. Morris, B. E. Willcox, A. Steinle, T. Spies and R. K. Strong: Complex structure of the activating immunoreceptor NKG2D and its MHC class I-like ligand MICA. *Nat Immunol* 2, 443-451 (2001)
41. H. G. Ljunggren and K. Karre: In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol Today* 11, 237-244 (1990)
42. M. B. Lodoen and L. L. Lanier: Viral modulation of NK cell immunity. *Nat Rev Microbiol* 3,59-69 (2005)
43. A. Marten, M. Lilienfeld-Toal, M. W. Buchler and J. Schmidt: Soluble MIC is elevated in the serum of patients with pancreatic carcinoma diminishing gammadelta T cell cytotoxicity. *Int J Cancer* 119, 2359-2365 (2006)
44. F. E. McCann, P. Eissmann, B. Onfelt, R. Leung and D. M. Davis: The activating NKG2D ligand MHC class I-related chain A transfers from target cells to NK cells in a manner that allows functional consequences. *J Immunol* 178, 3418-3426 (2007)
45. L. Mincheva-Nilsson, O. Nagaeva, T. Chen, U. Stendahl, J. Antsiferova, I. Mogren, J. Hernestal and V. Baranov: Placenta-derived soluble MHC class I chain-related molecules down-regulate NKG2D receptor on peripheral blood mononuclear cells during human pregnancy: a possible novel immune escape mechanism for fetal survival. *J Immunol* 176, 3585-3592 (2006)
46. A. Moretta, C. Bottino, M. Vitale, D. Pende, C. Cantoni, M. C. Mingari, R. Biassoni and L. Moretta: Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. *Annu Rev Immunol* 19, 197-223 (2001)
47. K. Ogasawara, J. A. Hamerman, H. Hsin, S. Chikuma, H. Bour-Jordan, T. Chen, T. Pertel, C. Carnaud, J. A. Bluestone and L. L. Lanier: Impairment of NK cell function by NKG2D modulation in NOD mice. *Immunity* 18, 41-51 (2003)
48. H. Onda, S. Ohkubo, Y. Shintani, K. Ogi, K. Kikuchi, H. Tanaka, K. Yamamoto, I. Tsuji, Y. Ishibashi, T. Yamada, C. Kitada, N. Suzuki, H. Sawada, O. Nishimura and M. Fujino: A novel secreted tumor antigen with a glycosylphosphatidylinositol-anchored structure ubiquitously expressed in human cancers. *Biochem Biophys Res Commun* 285, 235-243 (2001)
49. D. E. Oppenheim, S. J. Roberts, S. L. Clarke, R. Filler, J. M. Lewis, R. E. Tigelaar, M. Girardi and A. C. Hayday: Sustained localized expression of ligand for the activating NKG2D receptor impairs natural cytotoxicity *in vivo* and reduces tumor immunosurveillance. *Nat Immunol* 6, 928-937 (2005)
50. T. Osaki, H. Saito, T. Yoshikawa, S. Matsumoto, S. Tatebe, S. Tsujitani and M. Ikeguchi: Decreased NKG2D expression on CD8+ T cell is involved in immune evasion in patients with gastric cancer. *Clin Cancer Res* 13, 382-387 (2007)
51. G. Porcu-Buisson, M. Lambert, L. Lyonnet, A. Loundou, M. Gamerre, L. Camoin-Jau, F. Dignat-George, S. Caillat-Zucman and P. Paul: Soluble MHC Class I chain-related molecule serum levels are predictive markers of implantation failure and successful term pregnancies following IVF. *Hum Reprod* 22, 2261-2266 (2007)
52. S. Radaev, B. Rostro, A. G. Brooks, M. Colonna and P. D. Sun: Conformational plasticity revealed by the cocrystal structure of NKG2D and its class I MHC-like ligand ULBP3. *Immunity* 15, 1039-1049 (2001)
53. L. Raffaghello, I. Prigione, I. Airoldi, M. Camoriano, I. Levreri, C. Gambini, D. Pende, A. Steinle, S. Ferrone and V. Pistoia: Downregulation and/or release of NKG2D ligands as immune evasion strategy of human neuroblastoma. *Neoplasia* 6,558-568 (2004)
54. D. H. Raulet: Roles of the NKG2D immunoreceptor and its ligands. *Nat Rev Immunol* 3, 781-790 (2003)
55. D. H. Raulet: Interplay of natural killer cells and their receptors with the adaptive immune response. *Nat Immunol* 5, 996-1002 (2004)
56. V. Rebmann, P. Schutt, D. Brandhorst, B. Opalka, T. Moritz, M. R. Nowrousian and H. Grosse-Wilde: Soluble MICA as an independent prognostic factor for the overall survival and progression-free survival of multiple myeloma patients. *Clin Immunol* 123, 114-120 (2007)
57. J. Reinders, E. H. Rozemuller, K. J. van der Ven, S. Caillat-Zucman, P. J. Slootweg, R. A. de Weger and M. G. Tilanus: MHC class I chain-related gene a diversity in head and neck squamous cell carcinoma. *Hum Immunol* 67, 196-203 (2006)
58. M. Renedo, I. Arce, A. Rodriguez, M. Carretero, L. L. Lanier, M. Lopez-Botet and E. Fernandez-Ruiz: The human natural killer gene complex is located on chromosome 12p12-p13. *Immunogenetics* 46, 307-311 (1997)
59. P. Roda-Navarro, M. Vales-Gomez, S. E. Chisholm and H. T. Reyburn: Transfer of NKG2D and MICB at the cytotoxic NK cell immune synapse correlates with a reduction in NK cell cytotoxic function. *Proc Natl Acad Sci U S A* 103, 11258-11263 (2006)
60. H. R. Salih, H. Antropius, F. Gieseke, S. Z. Lutz, L. Kanz, H. G. Rammensee and A. Steinle: Functional expression and release of ligands for the activating immunoreceptor NKG2D in leukemia. *Blood* 102, 1389-1396 (2003)
61. H. R. Salih, D. Goehlsdorf and A. Steinle: Release of MICB molecules by tumor cells: mechanism and soluble MICB in sera of cancer patients. *Hum Immunol* 67, 188-195 (2006)
62. H. R. Salih, H. G. Rammensee and A. Steinle: Cutting edge: down-regulation of MICA on human tumors by proteolytic shedding. *J Immunol* 169, 4098-4102 (2002)
63. M. J. Smyth, J. Swann, E. Cretney, N. Zerafa, W. M. Yokoyama and Y. Hayakawa: NKG2D function protects

the host from tumor initiation. *J Exp Med* 202,583-588 (2005)
64. M. J. Smyth, J. Swann, J. M. Kelly, E. Cretney, W. M. Yokoyama, A. Diefenbach, T. J. Sayers and Y. Hayakawa: NKG2D recognition and perforin effector function mediate effective cytokine immunotherapy of cancer. *J Exp Med* 200, 1325-1335 (2004)
65. H. Song, J. Kim, D. Cosman and I. Choi: Soluble ULBP suppresses natural killer cell activity via down-regulating NKG2D expression. *Cell Immunol* 239, 22-30 (2006)
66. T. Spies: Induction of T cell alertness by bacterial colonization of intestinal epithelium. *Proc Natl Acad Sci U S A* 99, 2584-2586 (2002)
67. A. Steinle, P. Li, D. L. Morris, V. Groh, L. L. Lanier, R. K. Strong and T. Spies: Interactions of human NKG2D with its ligands MICA, MICB, and homologs of the mouse RAE-1 protein family. *Immunogenetics* 53, 279-287 (2001)
68. B. Suarez-Alvarez, A. Lopez-Vazquez, B. Diaz-Molina, M. J. Bernardo-Rodriguez, R. Alvarez-Lopez, D. Pascual, A. Astudillo, J. Martinez-Borra, J. L. Lambert, S. Gonzalez and C. Lopez-Larrea: The predictive value of soluble major histocompatibility complex class I chain-related molecule A (MICA) levels on heart allograft rejection. *Transplantation* 82, 354-361 (2006)
69. J. L. Upshaw, L. N. Arneson, R. A. Schoon, C. J. Dick, D. D. Billadeau and P. J. Leibson: NKG2D-mediated signaling requires a DAP10-bound Grb2-Vav1 intermediate and phosphatidylinositol-3-kinase in human natural killer cells. *Nat Immunol* 7,524-532 (2006)
70. C. S. Vetter, V. Groh, S. P. thor, T. Spies, E. B. Brocker and J. C. Becker: Expression of stress-induced MHC class I related chain molecules on human melanoma. *J Invest Dermatol* 118,600-605 (2002)
71. E. Vivier, E. Tomasello and P. Paul: Lymphocyte activation via NKG2D: towards a new paradigm in immune recognition? *Curr Opin Immunol* 14, 306-311 (2002)
72. I. Waldhauer and A. Steinle: Proteolytic release of soluble UL16-binding protein 2 from tumor cells. *Cancer Res* 66, 2520-2526 (2006)
73. N. F. Watson, I. Spendlove, Z. Madjd, R. McGilvray, A. R. Green, I. O. Ellis, J. H. Scholefield and L. G. Durrant: Expression of the stress-related MHC class I chain-related protein MICA is an indicator of good prognosis in colorectal cancer patients. *Int J Cancer* 118, 1445-1452 (2006)
74. S. A. Welte, C. Sinzger, S. Z. Lutz, H. Singh-Jasuja, K. L. Sampaio, U. Eknigk, H. G. Rammensee and A. Steinle: Selective intracellular retention of virally induced NKG2D ligands by the human cytomegalovirus UL16 glycoprotein. *Eur J Immunol* 33, 194-203 (2003)
75. K. Wiemann, H. W. Mittrucker, U. Feger, S. A. Welte, W. M. Yokoyama, T. Spies, H. G. Rammensee and A. Steinle: Systemic NKG2D down-regulation impairs NK and CD8 T cell responses *in vivo*. *J Immunol* 175, 720-729 (2005)
76. J. Wu and L. L. Lanier: Natural killer cells and cancer. *Adv Cancer Res* 90, 127-156 (2003)
77. J. Wu, Y. Song, A. B. Bakker, S. Bauer, T. Spies, L. L. Lanier and J. H. Phillips: An activating immunoreceptor complex formed by NKG2D and DAP10. *Science* 285, 730-732 (1999)
78. J. D. Wu, L. M. Higgins, A. Steinle, D. Cosman, K. Haugk and S. R. Plymate: Prevalent expression of the immunostimulatory MHC class I chain-related molecule is counteracted by shedding in prostate cancer. *J Clin Invest* 114,560-568 (2004)
79. W. M. Yokoyama and B. F. Plougastel: Immune functions encoded by the natural killer gene complex. *Nat Rev Immunol* 3, 304-316 (2003)
80. T. Zhang, A. Barber and C. L. Sentman: Generation of antitumor responses by genetic modification of primary human T cells with a chimeric NKG2D receptor. *Cancer Res* 66,5927-5933 (2006)

**Key Words:** NKG2D, MICA, NK cells, Tumor Immunosurveillance, Immunity, Immunology, Cancer, Tumor, Review

**Send correspondence to:** Dr Alexander Steinle, Interfakultaeres Institut fuer Zellbiologie, Abteilung Immunologie, Auf der Morgenstelle 15, 72076 Tuebingen, Germany, Tel.:49-7071-2980992, Fax: 49-7071-295653, E-mail: alexander.steinle@uni-tuebingen.de

**http://www.bioscience.org/current/vol13.htm**