

Natural Killer Cell Mediated Defence Directed Selectively Against Target Cells Lacking MHC Class I Gene Products *

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Introduction

Natural killer (NK) cells represent an important subset of lymphocytes. These cells fulfil crucial cytotoxic as well as regulatory functions of the immune system. Compromised NK cell activity has been found to be associated with the development of several diseases, including cancer, AIDS and virus infections. Intact NK cell activity appears to play an important role in health. More recent data suggests that NK cells may be involved in the pathogenesis of some human diseases and serve as an early predictor for susceptibility to disease [1]. In transplantation, NK cells participate in rejection of allogeneic bone marrow cells [2].

The molecular basis for the ability of NK cells to discriminate between normal and aberrant cells is not known in detail [3–5]. The aim of the present overview is to discuss studies of how target cell major histocompatibility complex (MHC) class I expression affects NK–target interactions and ultimately target susceptibility to NK cell mediated lysis.

The rationale for current studies is a hypothesis which has provided one test-

able model for self – non-self discrimination by NK cells [4, 5]. This hypothesis was originally based on (a) the patterns common to the various cytotoxic reactions attributed to NK cells and (b) a comparative analysis of the different strategies employed by vertebrates and invertebrates in order to distinguish between self and non-self. This hypothesis, presented as the “missing self” hypothesis [3], provided testable predictions for the investigation of the influence of the MHC class I gene products by NK cells. Briefly, it was suggested that NK cells could kill certain target cells because the latter express reduced levels of MHC class I molecules. This model underlies the work presented below. However, before going into the missing self hypothesis, present knowledge of NK cells and in particular their cytotoxic activity is briefly recapitulated.

Natural Killer Cells

The ability to kill certain tumour cell lines *in vitro*, without prior immunization or sensitization, was the first attribute of NK cells to be identified (reviewed in [6]). While this reaction was investigated in detail in the late 1970s several new insights as to their morphology, cell surface marker expression, tissue expression and action have become evident during the last 10 years (for reviews see e.g. [7–9]).

Morphologically, most NK cells are large granular lymphocytes (LGL). They are characterized by their intracytoplasmatic azurophilic granules and a high cytoplasm to nucleus ratio. In man,

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LGL comprise 2%–5% of peripheral blood lymphocytes. Early on, NK cells were found in the spleen peritoneal exudate and blood whereas they were scarcely found in lymph nodes, bone marrow, thoracic duct and the thymus. Later, NK cells were also isolated from the liver, tonsils, the epithelial lining of the upper respiratory tract and the lung interstitium [7–9].

NK cells commonly express certain cell surface markers, defined by monoclonal antibodies against CD 16 (the Fc γ III receptor for IgG) and NKH-1 in humans and NK-1.1/2.1 in mice [7–9]. Additional cell surface markers, some of which define subsets of NK cells, have recently been described [10]. NK cells are of bone marrow origin [6], but their exact lineage is uncertain. They share certain cell surface markers with T cells and also share some characteristics with monocytes. There is now a general belief that mature NK cells are distinct from T lymphocytes [6–10].

The T-cell antigen receptor is not involved in NK cell recognition or cytotoxicity [7–9]. NK cells do not express CD3. There is no rearrangement of alpha-, beta-, gamma-, or delta-T cell receptor (TCR) genes and no synthesis of functional TCR messenger ribonucleic acid (mRNA) (even though nonfunctional beta and gamma TCR transcripts may be detected). However, CD3 positive (alpha-beta or gamma-delta) T cells may express, particularly upon activation, MHC nonrestricted cytolytic activity against target cells that are also sensitive to NK cells. According to a new definition proposed at the 5th International Natural Killer Cell Workshop (Hilton Head, S.C., 1988) these cells should *not* be termed NK cells but rather T cells displaying “NK-like” activity or “non-MHC-requiring” cytotoxicity [7, 8].

NK cells also respond to various lymphokines and interferons by elevated cytotoxic activity. They have been reported to produce different regulatory lymphokines themselves, e.g. IL-1, IL-2, IL-4, IL-5, interferons and colony-

stimulating factors. Through such mediators (and maybe yet other unknown factors) NK cells are involved in regulation of haematopoiesis and lymphocyte functions [7–9].

Natural resistance to infectious agents may be one of the more important functions for NK cells in vivo [11, 12]. Particularly during virus infections, high levels of IFN (primarily alpha or beta), are induced in lymphoid organs. Interferons activate NK cells to a higher level of cytotoxicity and stimulate their blastogenesis and proliferation in vivo. Biron et al. [12] recently described a patient with a complete and persistent absence of NK cells but otherwise normal immune functions. This patient first presented with an overwhelming varicella virus infection requiring treatment with acyclovir and later a life-threatening cytomegalovirus (CMV) infection.

Graft Rejection Mediated by NK Cells

Soon after the discovery of NK cells it became clear that these cells could kill certain tumour cell lines in vitro in spite of the fact that they expressed no or only low amounts of MHC class I molecules [13, 14]. This was a significant difference from cytotoxic T cells, which require the presence of MHC molecules to specifically kill target cells [15]. Further, NK cells were shown to be the effectors in rejection of small numbers of certain transplanted tumour cells [6, 16], in the prevention of metastasis [17] and in bone marrow graft rejection [2, 18]. However, NK cells seem to have no or only little impact on established larger cancers. NK cells are not found within tumours; there is no clonal expansion, but rather a systemic suppression of NK activity [1].

A peculiar rejection mechanism, now attributed to NK cells, is the F₁-hybrid resistance [2, 19, 20]. This phenomenon has been instrumental in recognizing the in vivo activity of NK cells and it is of particular importance for the following

discussion. Hybrid resistance was first reported in 1958 when Snell [21] observed that homozygous lymphomas grew better in the strain of origin than in F_1 hybrids obtained by crossing this strain with another strain. This F_1 hybrid effect violated a principle in tissue transplantation. According to the genetic rules of histocompatibility, an F_1 hybrid should accept grafts from either of its parents. The most extensive genetic analysis of the F_1 hybrid effect was carried out by Cudkovic [in 20] who studied rejection of normal bone marrow cells in F_1 hosts. They explained the phenomenon on the basis of a positive recognition of hypothetical recessive Hh genes expressed in the parental (and on the graft) but suppressed in the F_1 animal [20]. Snell [22] later offered an alternative interpretation in which F_1 hybrid resistance was seen as a result of a combined match and mismatch rather than a complete match between host effector cells and transplanted cells. This theory originated before NK cells were known to be the effector mechanism in hybrid resistance but nevertheless formed the basis for the missing self hypothesis (developed below), providing an alternative explanation to the Hh model. Critical experiments demonstrating that NK cells were the effector mechanism in F_1 hybrid resistance were published in 1977 by Kiessling et al. [2]. Before that, Kiessling et al. [23] and Petranyi et al. [24] had reported a correlation between NK activity in vitro and tumour resistance in vivo among F_1 hybrids. Klein et al. [25] extended these conclusions and mapped F_1 hybrid resistance to the H-2 gene complex in several different tumour combinations. Carlson et al. [26] observed a rapid elimination of intravenously injected leukaemia cells whenever these were "mismatched", i.e. H-2K or D products were not present in relation to the host. This elimination occurred in nude mice but not in NK depleted mice.

Allogeneic lymphocyte cytotoxicity is a term used for the rapid destruction of intravenously injected allogeneic lym-

phocytes by unsensitized hosts. Allogeneic lymphocyte cytotoxicity has been reported in several mammalian species and it has been studied most extensively in rats [27]. It is mediated by NK cells and is in certain aspects related to F_1 hybrid resistance.

Strategies for Self–Non-self Discrimination: the Missing Self Hypothesis

Multicellular organisms need defence systems against destruction of their tissues by foreign invaders as well as against altered endogenous cells. A prerequisite for such a defence reaction is recognition of the potential threat. An organism should be able to discriminate between "self", i.e. everything constituting an integral part of a given individual, and the rest. This recognition could, in theory, be positive or negative [28]. In positive recognition, the organism actively recognizes "non-self" and reacts against it. In negative recognition, there is an active recognition of self and the reaction is triggered only as a consequence of the failure to recognize self. It is well known that higher vertebrates have evolved defence systems based on positive recognition. This is mediated by T and B cells which have clonally distributed receptors generated partly by a random process. The receptor repertoire is then somatically selected for the ability to positively identify "foreignness" either directly (B cells) or in the context of molecules of the MHC (T cells).

The missing self hypothesis is based on the second, negative type of recognition. It was suggested that NK cells kill certain targets because they fail to express adequate levels of self MHC class I gene products [3–5] (Table 1). This hypothesis originated from the observations that NK cells mediate rejection of allogeneic lymphoma and bone marrow grafts (H-2^{a/a} rejects H-2^{b/b}) and also, in contrast to cytotoxic T lymphocyte (CTL), F_1 -hybrid anti-parental resistance. In the

Table 1. Triggering signals for T cells and NK cells

Cell type	Strategy for self – non-self recognition	Signal
T cell	“Positive” recognition	Presence of a foreign antigen presented in the context of class I molecules of the MHC (triggering signal)
NK cell	“Negative” recognition	Presence of “self” class I molecules of the MHC (inhibitory signal) ^a

^a Note that this does not exclude other (MHC class I independent) triggering signals as well (see [3]).

allogeneic as well as in the F₁ hybrid anti-parental situation, the graft “fails” to express at least one H-2 class I allele of the host. This failure to express a complete set of self MHC class I molecule by the target was postulated to be sufficient to cause elimination by NK cells [4, 5].

Transposed to an autologous situation, the hypothesis predicted that absence or reduced expression of self MHC class I products (whether caused by mutation, transformation, arrest in differentiation or virus infection) could be sufficient to make a cell recognized and rejected by NK cells [4, 5]. Conversely, an induction of self MHC class I molecules on such a target cell should be sufficient to prevent rejection mediated by NK cells. These predictions have been tested *in vivo* and the results will briefly be discussed below.

Immunological Defence Against H-2 Class I Deficient Cells *in Vivo*

In order to test the missing self hypothesis we chose to work with the well-characterized C57Bl/6 derived RBL-5 and EL-4 murine lymphoma cell lines (H-2^b haplotype). These lymphomas express high levels of H-2 and are highly malignant in the syngeneic host. Our experimental approach was to test the prediction that selection for loss of H-2 class I expression should be accompanied by an increased sensitivity to natural resistance *in vivo* and *in vitro* [4, 5].

As a starting point, H-2 deficient variant lines were selected from the RBL-5 and EL-4 lymphomas [5, 29]. Titrated doses of wild type and variant cells were inoculated either subcutaneously [5, 29], intravenously or intraperitoneally [30] in small groups of mice, age matched and usually littermates, in several independent experiments and tumorigenicity was scored. This strategy minimized the risk that random fluctuations in the quality of the respective cell suspensions would be responsible for differences in the ability to form tumours.

In line with the prediction of the hypothesis, the H-2 class I deficient RBL-5 and EL-4 variants were rejected in syngeneic C57Bl/6 mice after a small tumour inoculum (10³ to 10⁵ cells), whereas mutagenized but non-selected, H-2 positive, wild type lines were highly tumorigenic in the corresponding doses [5, 29]. The H-2 deficient cells required a 10³ to 10⁴-fold higher dose than the H-2 positive cells to induce more than 50% tumour take irrespective if the cells were inoculated subcutaneously, intravenously or intraperitoneally [5, 29, 30].

The rejection of the H-2 deficient lines showed several characteristics of an NK mediated response, including thymus independence and no requirement for pre-immunization [5, 29]. The resistance was weakened (but not totally abrogated) by 400 rad irradiation [29], and it was sufficient to remove asialo-GM₁ or NK1.1 positive cells (NK cells) from the animal

to abrogate the rejection ([29], unpublished results). Experiments comparing the distribution and survival of isotope prelabeled variant and wild type cells indicated that a rapid elimination of the former took place within 24 h after intravenous injection. These differences in rapid elimination of tumour cells were abolished in NK depleted mice [29]. The above mentioned pattern was observed in all organs studied with one exception – the brain (discussed in detail in [30–32]).

One possible explanation for the differential rejection patterns of the H-2 positive and H-2 deficient lymphoma cell lines in the syngeneic B6 mice was a difference in an afferent arm of a NK dependent host response [33]. In this scenario the H-2 deficient cells would recruit NK effector cells which would kill H-2 positive and deficient cells equally well without discriminating between them. However, the differential rejection pattern remained when H-2 positive and deficient cells were inoculated into the same animals, whether in different flanks [29] or mixed in the same inoculum [33]. H-2 deficient cells were selectively eliminated even when they were present in a 10-fold excess compared to H-2 positive cells in the same inoculum [33]. These results suggested that the NK dependent response against H-2 deficient cells was selective in an efferent (effector) arm of the response [33].

In recent experiments we have demonstrated that it is possible to restore the tumorigenicity of β 2-microglobulin (β 2m) negative EL-4 cell lines by transfection of β 2m (R. Glas et al., to be published). This indicates that β 2m may act as a tumour growth promoting gene when the host defence is dominated by NK cells.

The “missing self” hypothesis predicted that it should be possible to obtain NK mediated rejection of a H-2 positive target provided that the host carried one (or several) extra MHC class I allele(s) in relation to the target. F₁ hybrid resistance and allogeneic lymphocyte rejection were postulated to be examples of this [4, 5]. To

directly test this concept, still within the RBL-5 model, we used the transgenic strain D8 generated by Bieberich et al. [34]. The D8 strain was produced by introducing an 8.0-kb genomic fragment containing the H-2D^d gene and 2.5 Kb 5' and 2.0 Kb 3' flanking sequences into B6 zygotes. The transgene product was expressed in different tissues in the same way as the endogenous H-2^b haplotype products, without alterations in the expression of the latter. Tumour growth was followed after subcutaneous inoculation of graded doses of RBL-5 lymphoma cells in D8 and B6 control mice [35]. The D8 strain was more resistant to subcutaneous challenge of “previously syngeneic” RBL-5 lymphoma cells than B6 controls. The direct role of the H-2D^d gene in this resistance was investigated by the use of (D8XB6)F₁ crosses and (D8XB6)XB6 backcrosses. The latter showed cosegregation with regard to the D^d expression and lymphoma resistance, both of which were inherited in a pattern consistent with control by a single dominant gene [35]. The resistance to RBL-5 (or other B6 derived lymphomas) in the D8 strain could be abrogated by pretreating mice with anti-asialo GM₁ antiserum or anti-NK 1.1 mAb, indicating that NK cells were necessary for the rejection [35]. Subsequent studies have shown that the elimination of RBL-5 in D8 is a rapid event taking place within 24 h (P. Höglund et al., J Exp Med, in press). In thus entirely resembles the elimination of RBL-5 H-2 deficient variant cells in B6 mice. A similar pattern was seen when the D8 strain was grafted with B6 bone marrow. The D8 recipients had acquired an ability to reject bone marrow from C57BL/6 donors but not from D8 donors and this rejection was dependent on the presence of NK cells in the host [18].

Recessive Hh antigens have been mapped to the D region of the H-2 complex, although rejection did not require expression of the dominant D locus product of the graft (reviewed in [20]). Our data do not address the role of putative Hh antigens at the tumour/graft

level. However, no transcripts have been detected from the flanking sequences of the D^d gene carried by the construct (G. Jay, unpublished observations). It is therefore concluded that the D^d gene itself is responsible for the resistance at the level of the host. These results are consistent with the predictions of the missing self hypothesis [3–5].

In conclusion, the results are in line with an NK cell mediated rejection of small tumour or bone marrow grafts providing that the graft lacks (or expresses greatly reduced levels) of at least one MHC class I allele of the host.

MHC Expression and Tumorigenicity – a Re-evaluation

The results obtained with the H-2 deficient lymphoma grafts in vivo predicted that if the critical immunological host–tumour interaction is dominated by NK cells rather than T cells in a given system, up-regulation of MHC class I expression would make tumour cells more malignant because they would survive interactions with NK effectors.

This has been observed in studies with different sublines of the mouse B16 melanoma, pioneered by Taniguchi et al. [37]. H-2 positive melanoma cells gave rise to a high number of metastatic lung colonies, whereas the H-2 low or deficient melanoma cells gave no or only few colonies after intravenous inoculation [37]. Sub-

sequent studies showed that the non-metastatic H-2 deficient sublines acquired metastatic capacity if they were pretreated with interferon (IFN), known to enhance H-2 class I expression, or if the mice were pretreated with anti-asialo GM1 serum, known to deplete NK cells [38].

In contrast to the result reviewed above, there are several reports in which decreased expression of MHC class I molecules are associated with enhanced tumour growth (reviewed in [39, 40]). The apparently conflicting results may depend on the antigenicity of the tumour and/or the experimental protocol used [3, 41]. This can be illustrated with the RBL-5 lymphoma, for which opposite results with regard to tumorigenicity were obtained when H-2 positive and deficient variants of this tumour were compared in two different experimental situations (Table 2):

- 1) A small subcutaneous inoculum in untreated mice led to growth of H-2 positive cells and NK dependent elimination of H-2 deficient cells, and
- 2) A large subcutaneous inoculum in pre-immunized mice resulted in T cell dependent elimination of H-2 positive cells and growth of H-2 deficient cells which overrode the limited non-adaptive NK response.

Had the tumours only been tested under the latter conditions, we would erroneously have concluded that loss of class I

Table 2. Influence of experimental protocol on NK versus T cell mediated rejection of RBL-5 H-2 positive and H-2 deficient sublines in B6 mice^a

RBL-5 tumour dose	H-2 expression	Pretreatment of the host	Progressive tumour growth
Low	High	–	Yes
Low	Low	–	No
High	High	–	Yes
High	Low	–	Yes
High	High	Preimmunization ^b	No
High	Low	Preimmunization	Yes

^a Data based on [5, 29, 33] and H. G. Ljunggren et al. (unpublished observations).

^b Preimmunized with RBL-5 (resembling grafting with a highly immunogenic tumour).

molecules is only associated with enhanced tumorigenicity of the tumour.

Thus, there is no obligatory association between reduced H-2 class I expression and increased malignancy. The effect of MHC class I expression on rejection or escape from immunological rejection will depend on the dominant host-tumour interaction. Such interactions may vary in different phases of tumour progression and under different experimental conditions [3, 41].

Tests for the effect of MHC modulation on tumour growth or immunotherapy therefore require careful experimental design to cover the action of different effector mechanisms *in vivo*. Since T cell responses, once elicited, would play a dominant role in the final outcome of tumour growth, the effect of H-2 changes on the NK defence could rather easily be missed in studies with relatively large tumour grafts and immunogenic tumours. This possibility can be controlled in any system, by using small grafts or short-term assays where rapid rejection of grafted cells is monitored, either in conventional transplantation assays or in tests of the survival of radiolabelled cells [29]. Additional controls can be obtained by the use of mice with either genetically inherited immunodeficiencies (e.g. nude mice or SCID mice) or mice where different subsets of the host immune defence experimentally has been depleted (e.g. irradiated, thymectomized, anti-NK1.1 treated, anti-asialo GM₁ treated, anti-L3T4 treated or anti-Ly2 treated mice).

NK Sensitivity of MHC Class I Deficient Cells In Vitro

A detailed review of the *in vitro* NK sensitivity of MHC class I deficient cells has recently been published [3]. A brief summary is given below.

The MHC class I deficient RBL-5 and EL-4 murine lymphoma cell lines, used in the *in vivo* studies described above, showed enhanced NK sensitivity *in vitro*

compared to their wild type counterparts [3, 5, 33]. The association between high NK sensitivity and reduced MHC class I expression in murine models is not confined to lymphoma or melanoma variants derived by mutagenization and selection *in vitro*. Fibrosarcoma clones with constitutively low H-2 expression derived by cloning without selection were sensitive to NK mediated lysis, while clones (from the same primary tumour) with high levels of H-2 expression were resistant [42].

Increased NK sensitivity of human MHC class I deficient variants was first reported by Harel-Bellan et al. [43] and Storkus et al. [44]. They analysed T-LCL, B-LCL and B and T cell hybrid cell lines. The latter group analysed three different sets of cloned cell lines with corresponding variants that differed in their relative HLA class I expression. While sensitivity correlated with reduced class I expression it did not correlate with class II expression or transferring receptor expression [44]. In one of two more recent confirmatory studies on NK sensitivity of HLA deficient variants [45, 46], IL-2 activated effectors gave the same MHC class I related pattern as fresh NK cells while allospecific CTL lines showed an opposite pattern [45]. The latter study also showed that an intermediate HLA class I expressor (Haplotype loss) variant was moderately NK sensitive (compare to the relatively resistant wild type line), whereas a weak HLA class I expressor (with an additional down regulation of the remaining haplotype) was highly sensitive [45].

Enhanced NK sensitivity of murine and human MHC class I deficient variant lines does not correlate with a single molecular defect (see [47]). Rather, it appears that increased NK sensitivity can result from different defects in the MHC class I biosynthesis with the common denominator that cell surface expression of class I molecules was impaired.

In order to directly study whether MHC class I gene products can affect NK sensitivity it is essential to study cell lines

transfected with different genes with the purpose to specifically restore the HLA/H-2 class I expression. The first studies in this direction did not support a role for MHC class I gene products. The murine line 1 carcinoma express little if any H-2D^d in vitro [48]. Transfection of H-2D^p into line 1 led to a constitutive and dimethyl sulphoxide (DMSO) inducible expression of H-2D^p but this expression had no influence on NK sensitivity. Transfection of H-2K^b into a hepatoma gave a less conclusive result (reduced NK sensitivity in 4/9 experiments, no effect in 5/9 experiments) [49]. The authors further concluded that there was no difference in tumour formation between wild type and transfected cells, and thus that H-2K^b did not affect either NK susceptibility or tumorigenicity. The latter conclusion was based on an i.m. inoculation of 10⁷ cells [49], as opposed to low dose inocula used to study rejection of H-2 deficient cells as reviewed in the present overview (Table 2).

However, when Quillet et al. [50] transfected the human β 2m gene into the β 2m negative human Burkitt lymphoma cell line Daudi, this led to the establishment of a line permanently expressing HLA-A10, -A11 and B-17 class I molecules. This transfected line showed a reduced sensitivity to both NK and lymphokine activated killer (LAK) cell lysis as compared to the HLA class I negative wild type cell line [50]. Transfection of the human HLA class I deficient lymphoblastoid B cell line C1R with HLA-A3, HLA-B7 and HLA-Bw58 also led to a reduction in NK sensitivity [51]. Although there was no significant variation among the HLA-A3, -A7 and -Bw58 alleles, HLA-A2 appeared unable to protect from NK cell lysis [51]. Comparison of amino acid sequence suggests a restricted number of residues which may be relevant to the protective effect. The protection did not extend to H-2D^p or K^b transfected C1R cells, nor was it seen when IL-2 stimulated NK effector cells were used [51]. Another B cell lymphoblastoid cell line, 0.221, with a selective

loss of HLA class I expression was transfected with HLA-A1, HLA-A2, HLA-B8, HLA-B5 or an HLA-C gene [46]. Expression of MHC class I genes reduced NK cell sensitivity, with a general tendency for HLA-B genes to have the most prominent effect.

Transfection of human melanoma cells with *c-myc* is associated with a specific down regulation of class I expression which is most prominent for the B locus products [52]. This is associated with an increased sensitivity to NK cell lysis [53]. Interestingly, this increased NK sensitivity can be overcome by super-transfection of HLA-B7 and B27 genes to the melanoma cells which restores the original NK resistant phenotype (P. Schrier, personal communication). The increased NK sensitivity of melanomas with a *c-myc* induced HLA-B suppression as well as some of the above-mentioned transfection studies suggested that selective down regulation of certain allelic or locus specific products (and not necessarily all class I molecules) might be sufficient to induce NK sensitivity. This is interesting in relation to two papers describing the failure to induce a significant NK resistance upon transfection with HLA-A2 [54, 55].

There are now two murine analogues to the rescue of HLA in β 2m transfected Daudi cells. Sturmhöfel and Hämmerling [56] selected an H-2 class I deficient line (S3) from the murine EL-4 cell line. This variant was found to have a defect in β 2m expression and was highly sensitive to NK cell lysis. Transfection of the S3 line with the β 2m clone restored cell surface H-2 expression and resulted in a considerable decrease in NK sensitivity. Transfection of class II genes had no effect. Blocking of class I molecules with Fab fragments against class I molecules increased NK sensitivity of EL-4 to the level of the S3 variant [56]. The second example is the transfection of the β 2m deficient YAC-1 variant A.H-2- with β 2m. This restored the YAC-1 phenotype with respect to inducible class I expression and a concomitant protection

from NK cell lysis after treatment with IFN-gamma [57]. The YAC-1 lymphoma has also recently been transfected with H-2K^b under control of the human metallothionein IIA promoter. These transfected H-2K^b positive sublines showed a reduced sensitivity to murine NK cell lysis [58].

At this stage it can be concluded that expression of certain MHC class I molecules can reduce NK sensitivity in several targets. Different molecular models for how MHC class I molecules can protect certain target cells as well as interpretations of the systems where MHC class I genes do not have this effect on NK sensitivity has recently been discussed (see [3]).

General Conclusions and Future Prospects

In the present overview we have discussed an NK cell defence against MHC class I deficient cells. The data in support of this concept were obtained with murine MHC class I deficient variant cell lines and their corresponding wild type cell lines in *in vivo* and *in vitro* studies. Using MHC class I transgenic mice, we have demonstrated that a deficiency of a single host MHC class I allelic product on a tumour cell graft is sufficient to cause rejection. $\beta 2m$, one of the three subunits of an MHC class I molecule [59, 60], is the first identified molecule that contributes to the IFN-gamma mediated protection from NK cell lysis [57]. This was suggested, although formally not proven, to be mediated through the increased cell surface expression of class I molecules of the MHC. The general concept of a surveillance system geared to detect "missing self" explains some conflicting results regarding the relationship between MHC class I expression, tumorigenicity and metastasis (see [3]). The concept can also explain F₁-hybrid resistance and rapid elimination of allogeneic lymphocytes by NK cells. Rapid elimination of allogeneic lymphocytes, transferred during for example pregnancy or sexual

contacts where the transferred cells may cause tissue damage by graft versus host or by transmitting infectious agents such as HIV, may be an important defence action by NK cells. From a more general immunological point of view the present results have challenged the notion that discrimination between self and non-self is the only strategy for immunological elimination of aberrant cells in mammals [3–5]. In relation to clinical bone marrow transplantation, the present defence system may be taken into consideration in discussions of host versus graft reactions [18].

The analysis of models where MHC class I molecules clearly do affect NK sensitivity must now focus on detailed molecular events. Experimental strategies for this analysis has recently been described [3]. The mechanism by which an NK cell recognizes a target cell, deficient in self MHC class I expression is unknown. A detailed analysis as to which parts of the MHC class I molecule contribute to the inhibition of NK cell lysis must be undertaken. Such an analysis may lead to insights into the recognition event. Other studies must focus on defining receptor-like structures on NK cells. In an "effector inhibition" model [3] certain specific questions could be asked. Does one of the NK receptors resemble a T cell receptor due to a possible MHC class I binding ability? Is such a receptor primed to recognize only presence of self MHC class I molecules, and does it trigger the lytic machinery if appropriate class I molecules are not recognized? It would also be of interest to know when during ontogeny NK cells learn to discriminate between self and non-self. Do NK cells themselves need to express self MHC class I in order to recognize absence of self MHC class I? Are quantitative levels of MHC class I on the target monitored relative to the MHC class I expression on the NK cell itself? The ultimate model for such and related studies may be $\beta 2m$ deficient mice as recently described in the literature [61, 62].

References

- Whiteside TL, Herberman RB (1989) The role of natural killer cells in human disease. *Clin Immunol Immunopathol* 53:1–23
- Kiessling R, Hochman PS, Haller O, Shearer GM, Wigzell H, Cudkowicz G (1977) Evidence for a similar or common mechanism for natural killer cell activity and resistance to hemopoietic grafts. *Eur J Immunol* 7:655–663
- Ljunggren HG, Kärre K (1990) In search of the missing self: MHC molecules and NK cell recognition. *Immunol Today* 11:237–244
- Kärre K (1985) Role of target histocompatibility antigens in regulation of natural killer activity: a reevaluation and a hypothesis. In: Herberman RB, Callewaert D (eds) *Mechanisms of cytotoxicity by NK cells*. Academic, New York, pp 81–91
- Kärre K, Ljunggren HG, Piontek G, Kiessling R (1986) Selective rejection of H-2 deficient lymphoma variants suggests alternative immune defence strategy. *Nature* 319:675–678
- Kiessling R, Wigzell H (1979) An analysis of murine NK cells as to structure, function and biological relevance. *Immunol Rev* 44:165–208
- Hercend T, Schmidt RE (1988) Characteristics and uses of natural killer cells. *Immunol Today* 9:291–293
- Ades EW, Lopez C (eds) (1989) *Natural killer cells and host defence*. Karger, Basel
- Trinchieri G (1989) Biology of natural killer cells. *Adv Immunol* 47:187–375
- Sentman CL, Hackett J, Kumar V, Bennet M (1989) Identification of a subset of murine natural killer cells that mediates rejection of Hh-1d but not Hh-1b bone marrow grafts. *J Exp Med* 170:191–202
- Welsh RM, Fitzgerald-Bocarsly P (1989) Anti microbial defence by NK cells: Chairmen's summary. In: Ades EW, Lopez C (eds) *Natural killer cells and host defence*. Karger, Basel, pp 105–107
- Biron CA, Byron KS, Sullivan JL (1989) Severe herpesvirus infection in an adolescent without natural killer cells. *N Engl J Med* 320:1731–1735
- Trinchieri G, Santoli D (1978) Anti-viral activity induced by culturing lymphocytes with tumor derived or virus-transformed cells. *J Exp Med* 147:1314–1333
- Stern P, Gidlund M, Örn A, Wigzell H (1980) Natural killer cells mediate lysis of embryonal carcinoma cells lacking MHC. *Nature* 285:341–342
- Yedell JW, Bennik JR (1990) The binary logic of antigen processing and presentation to T cells. *Cell* 62:203–206
- Kärre K, Klein GO, Kiessling R, Klein G, Roder JC (1980) Low natural in vivo resistance to syngeneic leukemias in natural killer-deficient mice. *Nature* 284:624–626
- Hanna N, Fidler IJ (1981) Relationship between metastatic potential and resistance to natural killer cell mediated cytotoxicity in three main tumor systems. *INCI* 66:1183–1190
- Öhlén C, Kling G, Höglund P, Hansson M, Scangos G, Bieberich C, Jay G, Kärre K (1989) Prevention of allogeneic bone marrow graft rejection by H-2 transgene in donor mice. *Science* 246:666–668
- Cudkowicz G, Bennet M (1971) Peculiar immunobiology of bone marrow allografts. II. Rejection of parental grafts by resistant F1 hybrid mice. *J Exp Med* 134:1513–1528
- Bennet M (1987) Biology and genetics of hybrid resistance. *Adv Immunol* 41:333–445
- Snell GD (1958) Histocompatibility genes of the mouse. II. Production and analysis of isogenic-resistant lines. I *Natl Cancer Inst* 21:843–877
- Snell GD (1976) Recognition structures determined by the H-2 complex. *Transplant Proc* 8:147–156
- Kiessling R, Petranyi G, Klein G, Wigzell H (1976) Non T cell resistance against a mouse Moloney lymphoma. *Int J Cancer* 17:275–281
- Petranyi G, Kiessling R, Povey S, Klein G, Hertzberg L, Wigzell H (1976) The genetic control of natural killer cell activity and its association with in vivo resistance against a Moloney isograft. *Immunogenetics* 3:15–28
- Klein GO, Klein G, Kiessling R, Kärre K (1978) H-2 associated control of natural cytotoxicity and hybrid resistance against RBL-5. *Immunogenetics* 6:561–569
- Carlson GA, Melnychuk D, Meeker MJ (1980) H-2 associated resistance to leukemia transplantation: natural killing in vivo. *Int J Cancer* 25:83–89

27. Heslop BF, Mcneilage LJ (1989) The F1 hybrid effect in allogeneic lymphocyte cytotoxicity. *Transplantation* 48:634–639
28. Klein J (1982) In: *Immunology – the science of self-nonsel self discrimination*. Wiley, New York
29. Ljunggren HG, Kärre K (1985) Host resistance directed selectively against H-2 deficient lymphoma variants: analysis of the mechanism. *J Exp Med* 162:1745–1759
30. Ljunggren HG, Yamasaki T, Collins P, Klein G, Kärre K (1988) Selective acceptance of MHC class I deficient tumor grafts in the brain. *J Exp Med* 167:730–735
31. Yamasaki T, Ljunggren HG, Öhlén C, Klein G, Kärre K (1989) Enhanced H-2 expression and T cell dependent rejection after intracerebral transplantation of the murine lymphoma YAC-1. *Cell Immunol* 120:387–395
32. Ljunggren HG, Yamasaki T, Collins VP, Kärre K (1990) Clearance of experimental tumor grafts in the central nervous system. Role of the major histocompatibility complex class I gene products. In: Johansson BB, Owman C, Widner H, (eds) *Pathophysiology of the blood brain barrier*. Elsevier, Amsterdam, pp 511–522
33. Ljunggren HG, Öhlén C, Höglund P, Yamasaki T, Klein G, Kärre K (1988) Afferent and efferent cellular interactions in natural resistance directed against MHC class I deficient tumor grafts. *J Immunol* 140:671–678
34. Bieberich C, Yoshioka T, Tanaka K, Jay G, Scangos G (1990) Functional expression of a heterologous major histocompatibility complex class I gene in transgenic mice. *Mol Cell Biol* 7:4003–4009
35. Höglund P, Ljunggren HG, Öhlen C, Ährlund-Richter L, Scangos G, Bieberich C, Jay G, Klein G, Kärre K (1988) Natural resistance against lymphoma conveyed by H-2Dd transgene to C57Bl mice. *J Exp Med* 168:1469–1474
36. Höglund P, Glas R, Öhlén C, Ljunggren HG, Kärre K (1991) Alteration of the natural killer repertoire in MHC transgenic mice. *J Exp Med* 174:327–334
37. Taniguchi K, Kärre K, Klein G (1985) Lung colonisation and metastasis by disseminated B16 melanoma cells: H-2 associated control at the level of the host and the tumor cell. *Int J Cancer* 36:503–510
38. Taniguchi K, Petersson M, Höglund P, Kiessling R, Klein G, Kärre K (1987) Interferon gamma induces lung colonization by intravenously inoculated B16 melanoma cells in parallel with enhanced expression of class I major histocompatibility complex antigens. *Proc Natl Acad Sci USA* 84:3405–3409
39. Doherty PC, Knowles BB, Wettstein PJ (1984) Immunological surveillance of tumors in the context of major histocompatibility complex restriction of T cell function. *Adv Cancer Res* 42:1–65
40. Bernards R (1987) Suppression of MHC gene expression in cancer cells. *Trends Genet* 3:298–301
41. Ljunggren HG, Kärre K (1986) Experimental strategies and interpretation in the analysis of changes in MHC gene expression during tumor progression. *J Immunogenet* 13:141–151
42. Algarra I, Öhlén C, Perez M, Ljunggren HG, Klein G, Garrido F, Kärre K (1989) NK sensitivity and lung clearance of MHC class I deficient cells within a heterogeneous fibrosarcoma. *Int J Cancer* 44:675–680
43. Harel-Bellan A, Quillet A, Marchiol C, Demars R, Tursz T, Fradelizi D (1986) Natural killer susceptibility of human cells may be regulated by genes in the HLA region on chromosome 6. *Proc Natl Acad Sci USA* 83:5688–5692
44. Storkus WJ, Howell DN, Salter RD, Dawson RD, Cresswell P (1987) NK susceptibility varies inversely with target cell class I HLA antigen expression. *J Immunol* 138:1657–1660
45. Öhlén C, Bejerano MT, Grönberg A, Torsteinsdottir S, Franksson L, Ljunggren HG, Klein E, Klein G, Kärre K (1989) Studies of sublines selected for loss of HLA expression from an EBV transformed lymphoblastoid cell line. *J Immunol* 142:3336–3341
46. Shimizu Y, Demars R (1989) Demonstration by class I gene transfer that reduced susceptibility of human cells to natural killer cell lysis is inversely correlated with HLA class I antigen expression. *Eur J Immunol* 19:447–452
47. Ljunggren HG, Pääbo S, Cochet M, Kling G, Kourilsky P, Kärre K (1989) Molecular analysis of H-2-deficient lymphoma cell lines. Distinct defects in biosynthesis and association of MHC class I heavy chains and β 2-microglobulin observed in cells

- with increased sensitivity to NK cell lysis. *J Immunol* 142:2911–2917
48. Dennert G, Landon C, Lord EM, Bahler DW, Frelinger JG (1988) Lysis of lung carcinoma by poly i:c-induced natural killer cells is independent of the expression of class I histocompatibility antigens. *J Immunol* 140:2472–2475
 49. Nishimura MI, Stroynowski I, Hood L, Ostrand-Rosenberg S (1988) H-2 Kb antigen expression has no effect on natural killer susceptibility and tumorigenicity of a murine hepatoma. *J Immunol* 141:4403–4409
 50. Quillet A, Presse FR, Marchiol-Fournigault C, Harel-Bellan A, Benbunan M, Ploegh H, Fradelizi D (1988) Increased resistance to non-MHC-restricted cytotoxicity related to HLA A, B expression. Direct demonstration using β 2-microglobulin transfected Daudi cells. *J Immunol* 141:17–20
 51. Storkus WJ, Alexander J, Payne JA, Dawson JR, Cresswell P (1989) Reversal of natural killing susceptibility in target cells expressing transfected class I HLA genes. *Proc Natl Acad Sci USA* 86:2361–2364
 52. Versteegh R, Kruse-Wolters K, Plomp AL, van Leeuwen A, Stam NJ, Ploegh HL, Ruiter DJ, Schrier PJ (1989) Suppression of class I human histocompatibility leukocyte antigen by c-myc is locus specific. *J Exp Med* 170:621–635
 53. Versteegh R, Pletenburg LTC, Plomp AC, Schrier P (1989) High expression of the c-myc oncogene renders melanoma cell prone to lysis by natural killer cells. *J Immunol* 143:4331–4337
 54. Stam NJ, Kast WM, Voordouw AC, Pastoors EB, van der Hoeven FA, Melief CJM, Ploegh HL (1989) Lack of correlation between levels of MHC class I antigens and susceptibility to lysis of small cellular lung carcinoma (SCLC) by natural killer cells. *J Immunol* 142:4113–4117
 55. Leiden JM, Karpinski BA, Gottschalk L, Kornbluth J (1989) Susceptibility to natural killer cell mediated cytotoxicity is independent of target cell class I HLA expression. *J Immunol* 142:2140–2147
 56. Sturmhöfel K, Hämmerling GJ (1990) Reconstitution of H-2 class I expression by gene transfection decreases susceptibility to natural killer cells of an EL-4 class I loss variant. *Eur J Immunol* 20:171–177
 57. Ljunggren HG, Sturmhöfel K, Wolpert E, Hämmerling GJ, Kärre K (1990) Transfection of β 2-microglobulin restores IFN mediated protection from natural killer cell lysis in YAC-1 lymphoma variants. *J Immunol* 145:380–386
 58. Carlow DA, Payne U, Hozumi N, Roder JC, Czitrom AA (1990) Class I (H-2 Kb) gene transfection reduces susceptibility of YAC-1 lymphoma targets to natural killer cells. *Eur J Immunol* 20:841–846
 59. Townsend A, Öhlén C, Bastin J, Ljunggren HG, Foster L, Kärre K (1989) Association of class I major histocompatibility heavy and light chains induced by viral peptides. *Nature* 340:443–448
 60. Ljunggren HG, Stam NJ, Öhlén C, Neefjes JJ, Höglund P, Hemmels MT, Bastin J, Schumacher TNM, Townsend A, Kärre K, Ploegh HL (1990) Empty MHC class I molecules come out in the cold. *Nature* 346:476–480
 61. Koller B, Smithies O (1989) Inactivating the β 2-microglobulin locus in mouse embryonic stem cells by homologous recombination. *Proc Natl Acad Sci USA* 86:8932–8935
 62. Zijlstra M, Li E, Sajjadi F, Subramami S, Jaenisch R (1989) Germ-line transmission of a disrupted β 2-microglobulin gene produced by homologous recombination in embryonic stem cells. *Nature* 342:435–438