Review of Literature

Cytotoxic lymphocytes constitute an important defence mechanism against transformed cells, virus infected cells, parasites and some invading microbes. Two types of lymphocytes participate in cytotoxic reactions. Cytotoxic T cells mediate an antigen specific, adaptive cytotoxicity response which is specifically generated in response to an antigenic challenge and takes several days to come up. In contrast, Natural killer (NK) lymphocytes do not require an induction and a certain level of NK activity pre-exists to act as a first line of defence against spontaneously arising tumor and certain viral infections. NK cells and cytotoxic T cells vary in their origin, maturation, differentiation pathways, phenotypes, mode of action, regulation kinetics of activation and target cell (TC) specificity. However, one thing common to both types of effectors is that they require a physical contact with the TC before initiating the killing process.

Natural Killer Cells: In the course of studies designed to identify the immune cytotoxic T cells in animals or patients with tumors, it was found that normal individuals without any apparent tumor load, also have lymohocytes with the ability to recognize and kill a variety of tumor cells (38). By 1975, it had become apparent that a class of lymphocytes which kill without the necessity of prior sensitization, exists naturally in the system (115). These lymphocytes were termed as the natural killer (NK) cells. Initially defined only by their spontaneous cytolytic activity, NK cells can now be unequivocally identified as a discrete subset of lymphocytes with distinct morphologic, immunologic and functional attributes. NK cells are bone marrow derived and are operationally defined by their ability to lyse a variety of target cells without prior sensitization (97). They are associated with the morphologically identified population of large granular lymphocytes (LGLs). LGLs comprise 5-10 % of the peripheral blood lymphocytes (PBLs). NK cells are found at highest

frequency in the peripheral blood (5-10 % of the peripheral blood mononuclear cells) and in the spleen (132). They are also found at lower frequency in the lung, liver, gastrointestinal tract and peripheral lymphoid organs (11, 120,131,143). While NK cells are bone marrow derived, the endogeneous levels of NK activity in bone marrow is low (88).

NK cells were first characterized as null cells (134) since they did not express markers characterestic of either B or T lymphocytes. Even now, there is no single marker which exclusively defines NK cells. Phenotypically, NK cells can be defined by a combination of markers. Human NK cells are predominantly $CD16^+$, $CD56^+$, $CD3^-$ and TcR^- (T cell receptor) (8,74). The CD16 and CD56 markers exhibit restricted distribution on peripheral blood lymphocytes and the combination $CD16^+$, $CD56^+$ serves as an accepted marker of NK cells. Other markers on the NK cells are CD2, CD7, CD11a, CD11b, CD18, CD38, CD45, CD45R, CD57 and the p75-Interleukin-2 (IL-2) receptor (74). Murine NK cells like their human counterparts, are also $CD3^-$, TcR^-, $CD16^+$ and in addition express asialo-GM1 ($ASGM1^+$) antigen. Mouse NK cells also express the allotypic antigens NK 1.1/NK 2.1 (12,101). Rat NK cells are $OX\text{-}8^+$ and $OX\text{-}9^-$ (93). None of these markers defining rodent NK cells are unique to NK cells and are cross reactive with T cells or monocytes (12,78). The absence of CD3-TcR complex on NK cells is important since, whereas TcR^+ T cells may mediate NK-like, major-histocompatibility complex (MHC) unrestricted killing, NK cell neither express nor require TcR for their cytotoxic activity (8,63,78,133). Morphological and immunological features of NK and cytotoxic T cells have been compared in Table- 1.

Lymphokine-Activated Killer (LAK) cells: In response to exogeneous activation stimuli such as IL-2, NK cells increase in size and become more granular. They acquire the ability to bind and kill a wider spectrum of tumor cell lines as well as fresh tumor cells which include both NK sensitive and NK resistant tumors (99). These IL-2 activated effector cells are generally

TABLE-1

A comparison between the characterestics of T cells and NK cells.

Character	T cell	NK cell
Morphology	Lymphocyte	Large granular lymphocyte
Origin	Bone marrow	Bone marrow
Thymic dependance for maturation	Yes	No
Size	Small	Large
Nucleus	Spherical	Indented
Cytoplasm	Clear	Contains azurophilic granules
Activity	Antigen/mitogen stimulated	Spontaneous
Memory	Present	Absent
MHC Restriction	Class I restricted	No restriction
Recognition structures	CD3-TcR complex	Not well defined
Phenotype	$CD3^+, TcR^+$	$CD3^-, TcR^-, CD16^+, CD56^+$

referred to as lymphokine-activated killer (LAK) cells and were originally described by Grimm et.al in 1982 (28).

In humans, LAK cell precursors cells have been shown to be $CD11^+$, $CD14^-$, $CD16^+$, Ia^-, Ig^- and $Thy\text{-}1^-$ while the effector LAK cells are $CD3^-$, $CD5^-$, $CD2^+$ and $CD16^+$ (118). Among the T-cells, a small subpopulation of $CD3^+$ $CD56^+$ lymphocytes (< 1% of the total T cells), may also generate LAK cells in response to IL-2 (56). In mouse, the progenitors of LAK cells were shown to be asialo- $GM1^+$, $Ly\text{-}2^-$, $L3T4^-$, Ia^-, Ig^- and $Thy\text{-}1^-$ (40,102). The phenotype of mouse LAK cells is also similar to that of activated NK cells [asialo-$GM1^+$, $Thy\text{-}1^+$, $CD16^+$ and Ia^-] (40,47).

LAK cells have an LGL morphology, characterised by an indented nucleus and abundant cytoplasm rich in azurophilic granules, which contain cytolysin and some enzymes possibly involved in the cytolytic process mediated by LAK cells. Like NK, LAK cells are a source of multiple cytokines. They release appreciable amounts of interferon gamma (IFN-g) and tumor necrosis factor-α (7).

Many cytokines are known to regulate the IL-2 activation of PBLs. IL-4 has a negative regulatory effect on IL-2 induced activation of peripheral blood and bone marrow NK cells, whereas it enhances the proliferation and cytotoxicity of IL-2 preactivated killer cells (73). IL-6 augments the cytotoxicity of human LAK cells (68). IL-7 has been shown to induce the formation of LAK cells from murine $CD8^+$ and $CD4^+$ T cells if they are separated from each other before exposure to IL-7 (125). IL-12 demonstrates a mitogenic effect on LAK cell blasts (26) and synergises with IL-2 in the generation of LAK cells (29,121). TNF is known to enhance the cytotoxicity of NK cells. Synergistic NK and LAK induction effects are seen when NK cells are incubated with TNF and low doses of IL-2 (82). Expression of IL-2 receptors is induced on $CD16^+$ LGLs by TNF alone and to a greater extent by the combination of TNF and IL-2 (81). Leukoregulin, a lymphokine secreted by LGLs, does not have any direct effect on effector LAK cells, but increases the target cell sensitivity to NK and LAK

cell mediated cytotoxicity (25).

Effector-target interactions: Cell mediated killing by immune cells requires contact between the effector and target cells. The surface contact may be initiated either by binding of the effector cell to antibody coated target cell through Fc receptors (ADCC), or through specific surface receptors on the effector cells. T cell receptor along with the CD3 molecule, on the cytotoxic T cell recognizes a specific target cell antigen in association with the target cell MHC I molecule. The process of cytolysis takes place in different stages which are similar to the NK-target cell interaction as described below.

The NK target cell (TC) interaction has several stages with the TC lysis being the final step in a cascade of events (39). The first step involves the recognition and binding of target structure(s) on the TC membrane (NK target structure-NKTS) by a recognition unit on the effector cell membrane. This conjugation is Mg^{+2} dependent and temperature insensitive (between 4°C and 37°C) (89,96). Initial cognate binding between NK effector cells and target cells, is further strengthened by energy-dependent and temperature sensitive interactions involving general adhesion/accessory molecules such as LFA-1 (94). This is followed by NK cell activation (triggering stage) which is a temperature sensitive metabolic process requiring Ca^{2+} (89,41). The TCs activate the release of NK cytotoxic factor(s) from the effector cell granules. A specific target cell membrane element, NK-inducing structure (NKIS), distinct from those involved in the initial NK target recognition and binding, seems to be responsible for the activation of the conjugated effector cells (140). Upon activation, NK cells release the contents of their granules, which include perforin/cytolysin and NKCF. In the final step of the reaction, which is independent of the effector cell, the lethal hit is delivered. This involves the alignment of the NK cell golgi apparatus and the microtubule organizing centre, towards the target cell, and the subsequent translocation of the NK granules

to the effector-target cell interface (16,55). Degranulation follows with the release of cytolytic factors within the NK-target cell interzonal space (15). Following degranulation, the NK cell recycles to repeat the cytolytic process, while the target cell is lysed. Therefore, besides having an appropriate NKTS, NK sensitive target must possess the ability to stimulate effector cells to release the required cytotoxic factor or deliver the lethal hit. Finally, in order to be lysed by NK cells, a TC must be sensitive to the lethal hit delivered by the NK cell. NK resistance of TCs could be due to a defect or absence in any of these traits.

Mechanism of LAK cell mediated cytotoxicity appears to be similar to that of NK cell mediated lysis. However, the killing of NK resistant tumors by LAK cells may represent the acquisition of a qualitatively broader recognition repertoire than that present in the unactivated NK cells. A second alternative is that LAK cells efficiently lyse TCs expressing low amounts of the appropriate NKTS . This property of LAK cells has led to its use in the immunotherapy of human malignancies (98,138).

Molecules involved in NK-target interactions: While the nature of recognition molecules on NK/LAK effector cells is not clear, several candidate molecule(s) have been proposed in this regard. Recently two families of receptor like surface molecules have been identified on NK cells. These families are genetically linked and share structural features, but they appear to have opposite effects on NK cell activity. Members of the first family which activate natural killing, include NK receptor-protein-1 (NKR-P1) on rat NK cells (17) and NK 1.1 on mouse NK cells (101). NKR-P1 was first identified on IL-2 activated rat NK cells by the MAb 3.2.3, which reacted specifically with rat NK cells (17). MAbs directed against NKR-P1 stimulates degranulation by NK cells. It also mediates redirected lysis. These findings suggest that members of the NKR-P1 gene family may serve as receptors on NK cells, but the structures on target cells recognized by NKR-P1 molecule have not yet been characterized. Second related family of

molecules comprises Ly-49 and possibly other similar molecules, which send a negative signal to NK cells. Ly-49 molecule which is considered to be a receptor for class I MHC antigens on target cells, has been discussed in a subsequent section of this review.

Extensive work is being done to characterize the NKTS. Biochemical studies have suggested that the NKTS is probably a glycoprotein. Roder et.al (95) described the isolation of glycoproteins of apparent molecular weights (M_r) 130, 160 and 240 ± 10 kDa under reducing conditions from NK sensitive cell lines such as YAC-1, MOLT-4 and K562. Similar studies by Obexer et.al (76) have shown the presence of glycoproteins of M_r 80, 120 and 200 kDa on NK sensitive lines such as K562 and MOLT-4. They have also shown that these molecules are probably involved only in the conjugate phase and not in the lytic phase. MM200 melanoma cells and Chang liver cells, which are NK sensitive, were found to shed soluble glycoprotein gp120-140 into culture media (145). All these glycoproteins were found to inhibit NK cytolysis specifically and not affect the T cell mediated cytotoxicity (145). Regardless of the actual glycoprotein identification, these studies support the existence of a target cell-surface structure(s) which is trypsin resistant, heat labile and crucial to the NK specific recognition of target cells.

Many structures at both the target and effector cell level have been proposed as having significant roles in the NK mediated recognition and lysis. While most of these have failed to represent NKTS, they may yet function in NK interactions. Some of them have been discussed here.

Laminin-like structures: NK/LGL synthesize and express laminin (LM) or LM like structures on their cell surfaces (42,113). The expression of this material increases with IL-2 induction of NK cells, paralleling enhanced cytolytic activity (43,113). NK sensitivity of tumor target cells has been directly correlated with target cell expression of LM receptors (61). Anti-LM antibody inhibits the NK lysis of NK sensitive targets like YAC-1 and K562.

The inhibition occurs at the effector cell level and does not affect the formation of NK-target cell conjugates, thereby indicating that these molecules may be participating in post-binding, activation events and LM/LM-like receptors may form NK triggering structures on the target cell membrane (61,43). Recently a 48 kDa (p48) protein, cross reactive with the $\beta 2$ subunit of LM was found to be expressed in low levels on resting NK cells. The expression of p48 increased on IL-2 activation of NK cells (44). While resting T cells and IL-2 activated $CD3^+$, $CD56^+$ cells do not express p48, both $CD3^-$, $CD56^+$ and $CD3^+$, $CD56^+$ LAK cells express high levels of p48 (112).

Fibronectin (FN): Rat NK cells synthesize and express FN or FN-like molecules on their cell surface, the levels of which increase upon NK activation (103). Anti FN antibody inhibits NK lysis of YAC-1 cells at a postbinding step at the effector cell level (103), but does not block the lysis of K562 cells by human NK cells (42,43). This indicates that FN represents an accessory molecule in some but not all target cell systems. It might also suggest that the use of these molecules is species dependent. Potential FN receptors expressed on human target cell membrane might include the VLA-3, VLA-4 or VLA-5 integrin molecules which exhibit a binding capacity for FN and exhibit a broad tissue distribution (37).

CD16/CD3-ζ chain: More than 80% of the NK cells mediate ADCC against antibody coated target cells (74,132). Recognition of such target cells is conferred by the CD16 (FcR III, low affinity receptor) molecule which is expressed at the NK cell surface. CD16 is the only IgG receptor on NK cells (60). In addition to aiding in ADCC, the CD16 molecular complex is capable of transducing activation signals into the NK cell (60). This is shown by the costimulatory activity of anti CD16 antibodies in NK activation and the ability of anti CD16 murine hybridomas (but not anti-CD2, 11a, 11b, 18, 45, 45R) to trigger NK cells and to be lysed by them

(58). The CD16 is expressed as a complex with one disulfide linked CD3-ζ chain homodimer (59). The complex also contains 80-90kDa and/or 12kDa proteins (4). The CD16 complex has been implicated as an NK receptor based on studies where anti CD16 antibodies were found to inhibit NK-mediated cytotoxicity (46). However, there exists a subset of $CD16^-$ NK cells which is deficient in ADCC function but which mediates normal NK activity (57,74).

p80-K562 ligand: Ortaldo et.al (79,80) described both a potential target cell ligand (NKTS) and its corresponding NK receptor. A monoclonal antibody (MAb 36) was generated against K562 cells which was shown to inhibit human NK-mediated conjugation and cytolysis of K562 cells, at the target cell level (79). Antiserum raised against the MAb 36 was found to inhibit NK-mediated conjugation and cytolysis of both K562 and MOLT-4 cells at the effector cell level (79). This polyclonal antiserum defines a specific 80 kDa glycoprotein which is distinct from CD2, CD8, CD11, CD16 and CD56. The biochemical characterization and cloning of p80 are yet to be done.

CD45: CD45 (leukocyte common antigen, T200) represents a family of molecular isoforms generated by alternate splicing of the same gene transcript (126). Human and mouse NK cells express the CD45 and CD45R molecules at their cell surface (114). Studies have shown that anti CD45 antibodies inhibit NK cytolysis at a post binding, Ca^{2+} independent stage at the effector cell level (84,119). The mode of anti CD45 inhibition of NK lysis is not well understood, but it may involve the modulation of NK receptor complex signal transduction. Signal modulation by CD45 molecules on T cells may involve CD45 mediated dephosphorylation of signal transducing or membrane associated phosphoproteins such as the CD3-ζ chain (62). Since NK cells also express the CD3-ζ chain this might prove a tenable explaination for CD45 inhibition of NK activity.

MHC class I antigens: Major Histocompatibility Complex (MHC) class I antigen is a polymorphic, multi-allelic, heterodimeric glycoprotein (53,136). The class I molecule is comprised of a 44 kDa MHC-encoded, membrane-anchored, heavy chain that noncovalently associates with the soluble 11 kDa non-MHC encoded beta-2-microglobulin light chain (53,54,136). Association of MHC I heavy chain and beta-2-microglobulin occurs soon after their biosynthesis in the endoplasmic reticulum and is a necessary but not sufficient event for cell expression of the MHC I heterodimer (54). MHC I molecules associate with and present endogeneous antigenic peptides to CTL. CTL are thus antigen and MHC I restricted in their reactivity (23,146). On the other hand, NK/LAK cells are MHC I unrestricted in their reactivity with their target cells (31,123). NK cells are cytotoxic against those MHC I negative target cells which are not susceptible to the T cell mediated cytotoxicity. Therefore NK-mediated immunity is complementary to T cell mediated immunity in the protection of individuals against tumor and virus infected cells (48). Unlike T cells, NK cells can lyse xenogenic target cells, though optimal NK reactivity occurs within an isologous system (32). Inspite of this MHC I nonrestriction, target cell class I MHC expression may have a profound effect in determining their sensitivity to NK/LAK cells, in at least some systems.

The role of MHC I antigens expressed on target cell surfaces in the regulation of NK and LAK sensitivity is unclear. Several recent reports have suggested an inverse correlation between the level of MHC I expression on the target cells and their susceptibility to NK/LAK lysis (20,36,49,72,105,106,110). Normal cell targets such as fetal BM or brain cells (35,144), prothymocytes (33) or late stage B cells which express little or no MHC I antigens are NK reactive (122). Conversely, NK resistant medullary thymocytes, as well as other NK resitant normal cell types, show the opposite pattern (34). A similar inverse correlation was also found in various tumor cells including rat thyroid carcinoma (14), murine RCT sarcoma (70), B16 melanoma

(51), human fibrosarcoma (2), rat colon carcinoma (10) and in tumor mutants such as human B lymphoblastoid, EBV-transformed B-cell, murine RBL-2 and EL-4 lymphoma (2,77). These mutants are MHC I^- and exhibit elevated sensitivity to NK cell mediated cytotoxicity (NK-CMC) compared to their parental cells. This correlation extends to the *in vivo* tumor target systems in which NK is believed to be relevant. Primary or solid tumors, which are poorly regulated by NK *in vivo*, do not show a correlation between class I antigen expression and NK sensitivity (67,135). However, a significant correlation exists for the metastases which form targets for NK lysis *in vivo* (51,67,129). *in vivo* studies show that MHC I^- variants of a highly malignant MHC I^+ lymphoma are selectively rejected by the syngeneic host, probably by NK activity (51).

YAC-1 lymphoma cell grown *in vivo* as an ascites tumor express elevated MHC I level and show a concomitant decline in NK/LAK susceptibility (and enhanced sensitivity to CTL) compared to their *in vitro* grown counterparts. Similarly, B16 melanoma cells which are MHC I^+ and NK-resistant, when grown *in vitro*, show a reduction in their MHC I levels and a corresponding increase in their NK sensitivity (87,130).

It is not clear, whether NK cells specifically sense class I MHC molecules on target cells. Evidence for a MHC I sensing mechanism has however, been obtained recently. A second family of NK receptors, related to NKR-P1 family (discussed above), is represented by mouse Ly-49 which can interact with target MHC I molecules. In contrast to the NKR-P1, Ly-49 delivers inhibitory signals to the NK cells (50). A 85KDa, disulphide linked homodimer, is expressed by 20% of the $CD3^-$ NK cells in C57/BL6 mice (50). Karlhofer et.al (50) have shown that Ly-49^+ NK cells do not lyse target cells which are homozygous or heterozygous (50) with H-2^b) for H-2^d or H-2^k haplotypes. However, effector cells which are Ly-49^- spontaneously lyse these cells. The only exceptions to this were YAC-1 (H-2^a, which is H-$2D^d$ and H-$2K^k$) cells which are sensitive to both Ly-49^+ and Ly-49^- subsets, WEHI-3

and BB88 (H-2^d) cells which were resistant to both the subsets. Ly-49^+ IL-2 activated NK cells were unable to lyse H-$2D^d$ expressing targets by other mechanisms such as ADCC, MAb induced redirected lysis and lectin induced lysis also. This suggests that interaction of Ly-49 with an allo MHC class I antigen of either of the specified haplotypes negatively affects NK stimulation. This indicates that NK cells may possess inhibitory receptors that specifically recognize MHC I antigens.

Effects of experimentally manipulated MHC I expression on NK/LAK susceptibility:

(a) Differentiation Inducing Agents:- Treatment of target cells with agents such as tetradecanoyl phorbol acetate (TPA) (78), dimethyl sulfoxide (DMSO) (142) or sodium butyrate (141) resulted in increased MHC I expression, CTL sensitivity and NK/LAK resistance compared to their untreated counterparts. This effect may account for the relationship between state of target cell differentiation and sensitivity to NK reported for various cell lineages (65,78).

(b) Viral Infections:- Viral infection of target cells can result in reduced MHC I expression, as seen for infections by adeno virus, herpes virus and vesicular stomatitis virus. Reduction in MHC I levels in these infected cells confers resistance to $CD8^+$ CTL lysis (3,45) but enhances sensitivity to NK-CMC (100). The physiologic relevance of this host protection mechanism is under scored by the severe herpes virus infection that occur in patients exhibiting a selective NK deficiency (9).

(c) Regulation by Oncogenes:- Deregulation of cellular oncogenes have been shown to affect the class I antigen-NK sensitivity balance. Elevated c-myc or N-myc levels have been associated with a MHC I deficient, NK sensitive phenotype (6,137). Neuroblastomas which express amplified N-myc levels are class I deficient and NK sensitive. They have been reported to function efficiently as cold

target inhibitors of NK sensitive tumors such as K562 and MOLT-4 (69). Transfection of c-myc into melanoma cell results in tranfectants with diminished class I antigen levels and increased NK sensitivity (137). If class I levels are rescued by interferon treatment, NK sensitivity declines.

(d) Mutagenesis and Transfection:- In three independent human B lymphoblastoid cell line (B-LCL) systems, deletion of target cell class I genes by gamma irradiation followed by immunoselection with anti-class I monoclonal reagents results in class I-loss mutants (36,123). These mutants also exhibit increased sensitivity to NK lysis (36,77). Subsequent transfection of HLA class I genomic clones into these mutants generates transfectants which are high expressors of class I antigens and NK resistant (116,124).

Transfection of a functional beta-2-microglobulin gene into Daudi cells, which are deficient in cell surface expression of class I due to a genetic defect in beta-2 microglobulin translation (90), results in a class I^+, NK-resistant tranfectant (90). Similar observations were made following transfection of class I MHC gene into other class I^- target cells (30,127).

(e) Cytokines :- IFNs are a heterogeneous family of proteins. Type I IFN is induced in response to viral infections or bacterial stimulation and type II or immune IFN is induced in response to specific antigens, mitogens or other stimuli. Two species of viral IFN, IFN-a and IFN-b are the predominant forms produced by leukocytes and fibroblasts, respectively while immune IFN or IFN-g is the predominant form produced by stimulated lymphocytes. Besides the antiviral property, IFNs also exhibit anti-growth activity (86) and modulate cellular differentiation (86). They are known to stimulate the cytotoxic activities of lymphocytes, macrophages and natural killer cells (86). A major effect of IFNs is their modulation of expression of MHC antigens. All IFNs induce an increase in the surface expression of class I MHC antigens,

whereas class II antigens are stimulated predominantly by IFN-g with little or no effect by IFN a or b (22,86). Expression of Fc receptors is also stimulated by the IFNs (22,86).

Other cytokines such as TNF or IL-1 also result in an enhanced MHC I expression on the target cells. This leads to an increased sensitivity to $CD8^+$ CTL, but resistance to NK cells. Immature cells, variant cells, and certain virus infected cells with low MHC I expression despite stimulation by cytokines are a potential threat because they may escape T cell recognition even if they express foreign antigens. NK cells may function *in vivo* as a physiological surveillance system to selectively eliminate such cells. Thus, both NK and CTL cytotoxic arms may be regulated in part, by cytokine effects on pathways affecting target cells MHC I antigens. MHC variants of YAC-1, B16 melanoma, EL-4 thymoma, and transformed B cell lines which do not express MHC I antigens after treatment with IFN-gamma also failed to become NK and/or LAK resistant. However, they exhibited several other IFN- induced responses such as protection from virus infection, modulation of Con A capping, and inhibition of cell proliferation (36,127). In another set of experiments, IFN-gamma pretreated K562 cells were separated into MHC I^+ and MHC I^- subsets for NK assays. Both the populations were found to bind to effector cells with equal efficiency, but only the MHC I^+ subset showed a reduced sensitivity to NK lysis (91).

A similar correlation was seen following pretreatment of target cells with other cytokines. YAC cells treated with NK-LRIF (NK-lysis resistance inducing factor), which is purified from supernatants of Con-A stimulated spleen cell cultures, showed a marked enhancement in the expression of MHC I antigens and a decline in NK sensitivity (110). TNF also enhances the target surface MHC I levels and reduces NK susceptibility (83).

A direct casual link between MHC I expression and NK/LAK sensitivity has not been firmly established. Although cytokine induced (especially IFN induced) MHC I expression is often correlated with resistance to NK cells, other studies found no

correlation between these two activities (21,139). IFN alpha can protect Daudi cells and IFN gamma protects solid brain tumor cells from NK cells without inducing MHC I antigens (85). IFN treatment of BL6 melanoma (H-2^b- negative or positive sublines) resulted in NK resistance despite a dramatic difference in MHC I antigen levels (31). NK sensitive colon carcinoma PROb, glioma and neuroblastoma cell lines on treatment with IFN-g show an increase in their MHC I levels but no change in their NK/LAK susceptibility (10).

The mechanism by which class I molecules exert a protective influence on target cell sensitivity to NK is not known, although several models have been proposed (49,123). In the "missing self" hypothesis, Ljunggren and Karre have suggested that NK cells kill targets with low amounts of MHC I molecules because of reduced expression of "self" MHC I gene products (66). This hypothesis has its origins in the observations of NK cell mediated rejection of allogenic lymphoma and bone marrow grafts and F1-hybrid anti-parental resistance (52). Hence, this model predicted that absence or reduced expression of "self" MHC I products, caused either by mutation, transformation, arrest in differentiation or viral infection could be sufficient to allow a cell to be recognized and rejected by NK cells. Ljunggren and Karre (66) have also suggested the existence of multiple receptors on the NK cells which might be using different recognition strategies. One of these strategies may be employing the use of MHC I molecules. This would explain why several investigators, using different systems have found a lack of correlation between the surface MHC I levels and susceptibility to NK lysis. Two murine lymphoma cell lines, both expressing high levels of MHC I antigens, differ greatly in their sensitivity to NK cells (18). The human MHC I- cell lines Daudi and K562 are differentially susceptible to NK lysis, the former being resistant and the later being sensitive. Karre et.al and Harel-Bellan et.al have suggested that target cell expressed class I antigens represent an "off-signal" or "good-health signal" to the NK effector cell (36). Target cells exhibiting reduced

class I levels would be perceived to be in "bad health" and would be NK sensitive. This model however, cannot account for class I$^-$, NK resistant target cell such as astrocytes or primary and solid tumors.

The existence of a second, class I-independent molecule(s) that function as an NK target structure(s) has been postulated as an alternate model. This model postulates that class I and NKTS molecules associate on the target cell membrane, and the associated forms of NKTS are inefficiently recognized by NK cells. Thus class I protection could result from class I molecules masking or inducing conformational changes in associated NKTS. While several cell surface proteins such as class II antigens, IL-2 receptors as well as receptors for several growth factors associate with class I molecules, there is no direct evidence to support or refute this model.

The exact stage at which class I molecules interfere with NK activity is also debatable. While several studies suggest the protection to be at the level of effector-target cell conjugation (123,124), others refute this and suggest an effect on a post-binding stage of NK programming (36,90). It is also likely that NK cells interact with MHC I-associated molecules or molecular moieties coordinately regulated with MHC I. MHC I is found to associate with insulin-receptor and epidermal growth factor receptor. These molecules might contribute to the presentation of an inhibitory signal, thus explaining the inverse correlation between MHC I expression and NK sensitivity (87). The reported inhibitory action of MHC I antigens on effector-target conjugation does not preclude additional MHC I protective effects on a post-binding event. An increase in the expression of adhesion molecules is expected to compensate the suppressive effect MHC I molecules might have on the NK conjugation with the target cells. However, as seen in the case of IFN induced resistance, where an increase in adhesion molecules (ICAM-1, LFA- 1 and LFA-3) as well as MHC I molecules is seen, the MHC I molecules could still affect a postbinding event and confer protection against NK lysis.

Most of the work done to study the basis of MHC non-restricted lysis has been with the NK system. The LAK cells which are also MHC non-restricted exhibit a much wider target cell range. This might make the lytic mechanism used by LAK cells somewhat different from that of the NK cells. Studies are being done to characterize the molecules involved in LAK cell mediated cytotoxicity. Bean et.al (5) have reported diverse structures involved in the recognition phenomenon by evaluating the effects of treating effector and target cells with trypsin and chymotrypsin, enzymes that disrupt surface proteins. Trypsin and chymotrypsin treatments modified the B16 targets and inhibited LAK mediated killing, while the antigen being used on P815 targets for lysis was not affected by either of these enzymes, and the cytotoxicity of YAC-1 cells was affected only by chymotrypsin. The effect of enzyme treatment of YAC-1 cells on lysis by NK cells showed that, as opposed to LAK cells, trypsin treatment of YAC cells did affect killing by NK cells. When the LAK cells were subjected to enzyme treatment and the resulting effect on their cytotoxicity was studied, it was seen that trypsin treatment of LAK cells reduced their ability to lyse YAC- 1 cells, while NK lysis of YAC cells was reduced by both the enzymes. B16 cell lysis was inhibited by using trypsin as well as chymotrypsin treated LAK cells. These findings suggest the presence of a heterogenous group of molecules, receptors and target antigens, mediating the LAK cytotoxic phenomena.

The role of target MHC I antigens in regulating LAK sensitivity is debatable. Wiebke et.al (139) have reported that a clear cut correlaton between enhanced class I MHC antigen expression and decreased LAK susceptibility was not observed in fresh uncultured human melanomas. Similar results were also reported by De Fries and Golub (21), who observed that LAK susceptibility of S4 (human sarcoma) and M14 (human melanoma), following interferon treatment is not dependent on increased class I MHC antigen expression. However, by depleting class I MHC antigen expression on interferon treated human gliosarcoma cells,

Miyatake et.al (71) reported that interferon induced resistance to LAK lysis is, at least in part, due to enhanced levels of class I MHC antigen expression. Work from our laboratory has also demonstrated interferon induced LAK resistance in some murine tumor cell lines, which is inversely correlated with changes in the class I MHC antigen levels (105,106).

In the present study, we have made a detailed investigation into the role of target cell class I MHC antigen levels in determining its LAK susceptibility. We have chosen a panel of five murine and five human tumor cell lines and examined (a) correlation of their basal levels of class I MHC antigen with their basal LAK susceptibilities, (b) the effect of interferon induced upregulation of class I MHC antigen and selective abrogation of class I MHC antigens by a brief exposure to pH 3.0 buffer, on their LAK susceptibilities, (c) ability of all tumor cell lines to competitively inhibit the lysis of other tumor targets by LAK cells and (d) the effect of positive or negative modulation of class I MHC antigens on the ability of tumor cells to perform in cold target competition assays.

Current debate on the mechanism by which class I MHC molecules on target cells, influence the interaction between NK/LAK effector and target cells, has been re-examined in light of the data we have obtained.