Cytotoxic Cellular Mediators of the Immune Response to Neoplasia: A Review

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Immunotherapy in the management of neoplastic disease has recently been a major focus of scientific attention. Studies *in vitro* and in animal systems have provided the basis for the first trials of cellular immunotherapy for neoplasia in humans. Work over the past ten years has identified several distinct populations of lymphocytes active in lysing neoplastic cells, including major histocompatibility complex (MHC)-restricted and non-restricted cytotoxic T lymphocytes (CTL), natural killer (NK) cells, the natural cell-mediated cytotoxicity (NCMC) population, and the lymphokine-activated killer (LAK) phenomenon. This paper reviews the current understanding of the distinguishing cell surface phenotypes, recognition structures, mechanisms of neoplastic target cell lysis, activation requirements, and ontogeny of each of these cell groups.

INTRODUCTION

The role of the immune system in protection against neoplasia is becoming increasingly well-defined. While known to involve humoral responses, as in reported host generation of antibodies to malignant melanoma cells [1], cell-mediated responses have been the most extensively studied component in this protection. The cytolytic cell types mediating these responses fall into two broad categories: the specific, inducible group, which includes MHC-restricted CTLs, and the nonspecific innate group which includes natural killer (NK) cells, major histocompatibility complex (MHC) non-restricted cytotoxic T lymphocytes (CTLs), the natural cell-mediated toxicity (NCMC) population, and the lymphokine-activated killer (LAK) phenomenon. Action of the inducible cellular response is specific for neoplastic cell surface antigen, requires priming with induction of immunologic memory, and is mediated by MHC-restricted CTLs acting via the T-cell receptor (TcR). Action of the innate group is MHC non-restricted, characterized by a broad rather than a specific recognition repertoire, and does not participate in generation of immunologic memory. Studies with these cytolytic cells have provided the basis for the first trials of cellular immunotherapy for neoplasia in humans, performed over the past year in a multicenter study under the direction of the National Institutes of Health [2]. Much recent work on the immunologic response to neoplasia has focused on the cell surface phenotypes, recognition structures, mechanism of neoplastic cell lysis, activation requirements, and ontogeny of each of the effector cell groups involved.

TABLE 1
Partial List of T-Cell Surface Markers

Cluster of Differentiation (CD) Nomenclature	Previous Nomenclature	Murine Nomenclature
CD5	T1	
CD3	T3, UCHT-1	T3
CD8	T8, Leu2	Lty-2
CD4	T4, Leu3	L3T4
CD16	FcR[a]	FCR[a]
CD2	T11, Leu5	

[a]Fc receptor for immunoglobulin

MHC-RESTRICTED CTLs

These lymphocytes of the T-cell lineage, which appear to see specific tumor antigens in an MHC-restricted manner [3], have been implicated in host resistance to neoplasms such as certain sarcomas [1], plasmacytomas, and leukemias [3] and have been proposed for adoptive immunotherapy [4,5]. Surface markers in human MHC-restricted classical CTLs include:

Thy-1, Lyt-1, T3, TcR, Qa-2, Qa-5, Lyt-2, or L3T4   Murine
CD2, CD3, CD7, CD8, or CD4   Human

Nomenclature of T-cell surface markers relevant to these molecules is currently in transition and a partial list of relevant terms is presented in Table 1 for the sake of clarification.

There are at least three major T-cell subsets: cytotoxic T lymphocytes (CTLs) and the two regulatory cell types, T helper and T suppressor cells. The suppressor subset may not employ the TcR and is not relevant to the present discussion, but the CTLs and T helpers definitely function via the TcR and are the classical mediators of specific T-cell recognition in both neoplasia and in the other diverse roles of the specific immune response. The events in this process are mediated by the TcR protein structure with exquisite binding affinity for its specific antigen in the presence of MHC. This TcR recognition mechanism is applied throughout the immune system from the complex regulation of immunoglobulin secretion to the detection and lysis of virally infected cells.

The T-Cell Receptor (TcR) Complex

The TcR has recently been identified and its genes cloned and sequenced. There are three rearranging gene families, α, β, and γ, that can be potentially expressed in T cells. The α [6], β [7], and γ [8] genes have all been characterized in the past three years. These are homologous to immunoglobulin genes in that both TcR and immunoglobulin gene families have variable (V) regions, constant (C) regions, and joining regions (D and J regions). Mechanisms of generating TcR diversity have been shown to include combinatorial joining variety, junctional diversity, and combinatorial chain association [9]. It is this diversity that confers the ability for specific recognition of a virtually limitless universe of antigens, including those that arise on neoplastic tissues. The functional TcR consists of a disulfide-linked heterodimer composed of α and β gene products. These α and β chains are glycoproteins of approximately 43,000

daltons each and are non-covalently linked to several other molecules [10,11], which include the CD3 molecule in humans and several possible accessory molecules.

The CD3 molecule is composed of three polypeptides, δ, ϵ, and γ, of 21,000, 26,000, and 25,000 daltons, respectively [12]. CD3 appears to be closely associated with the TcR in that mutations that prevent the appearance of the α-β heterodimer also prevent the appearance of CD3 [13]. Its major functional role seems to involve activation of the T cell through transmembrane signalling after the TcR is bound [14]. Brenner and others have recently isolated γ gene products as well as a protein structure known as the δ chain on the surface of CD3($+$), α and β heterodimer($-$) T cells from immunodeficient patients [15].

The scope of function of this alternate TcR is currently unknown, but the high level of γ gene expression in immature Stage I thymocytes suggests a role in the ontogeny of mature T cells, and its recent identification as the recognition structure on a cloned line of MHC non-restricted CTLs [16] suggests involvement in MHC non-restricted recognition. The two major accessory molecules of TcR are CD4 and CD8. CD4 is a 62,000 molecular-weight molecule believed to be associated with the TcR on class II MHC-restricted helper T cells [17]. The major functional role of CD4 appears to be associated with MHC-restricted "help" and induction of activation of B and other T cells. The other major accessory molecule, CD8, is a 35,000 molecular-weight structure associated with class I MHC-restricted recognition and cytotoxic function [18]. The TcR complex, then, is composed of a clonally distributed heterodimer, CD3, and various accessory molecules. The exact role that each component plays in T-cell recognition is becoming increasingly clear.

MHC-restricted CTLs utilize this TcR structure to recognize neoplastic cells. Once recognized, the neoplastic cell is lysed by the CTL through two possible mechanisms, pore-forming proteins (PFPs) and toxic soluble mediators. PFPs from CTLs exhibit remarkable homology to C9 of the complement cascade and are believed to act by inserting into target cell membrane and forming a large aqueous pore which induces cell death by destruction of selective permeability. The molecular weight of PFPs is 70,000 daltons, and in the presence of calcium ions they will form pores 160Å in diameter [19]. Toxic soluble mediators include tumor necrosis factor (TNF) [20] and lymphotoxin (LT) [21]. Although the precise mechanisms of action of these soluble mediators remain to be determined, a common end-point appears to involve DNA fragmentation of the target cell [22]. Lytic activity via both mechanisms can be augmented by the presence of interleukin-2 (IL-2).

Ontogeny of CTLs

What is the ontogeny of this group of cytolytic effectors active against neoplastic cells? All T cells arise from a common precursor and are thymically dependent for differentiation [23]. Expression of surface molecules and transcription of TcR genes in T-cell maturation is shown in Table 2.

MHC-restricted CTLs, then, are T cells that help mediate the specific and inducible branch of the cellular immune response against neoplasia. They share surface markers, recognition mechanisms, activation requirements, and ontogeny with classical T cells and may have future potential in specific immunotherapy of neoplastic disease.

MHC NON-RESTRICTED CTLs

The existence of a population of T cells that recognize their targets in an MHC non-restricted manner as a distinct set of host effectors against neoplastic disease has

TABLE 2
T-Cell Ontogeny: Surface Markers and TcR mRNA Expression

	Stage I	Stage II	Stage III
Markers	CD11	CD11, CD1, DC4, or CD8	CD4, CD11, CD3, TcR
			or
			CD8, CD11, CD3, TcR
TcR gene (mRNA expression)	γ	γ, β	T helper: α, β
			CTL: α, β, γ

only recently been appreciated [24,25]. Initial support came from studies *in vitro* where cultured T cells were found to exhibit MHC non-restricted NK-like activity [26]. This finding was later confirmed by work *in vivo*, where T cells lysing neoplastic cells in an MHC non-restricted manner were found in circulating peripheral blood lymphocytes (PBL) [27] and as an element of the LAK phenomenon [28]. These CTLs have been shown to be active against such neoplasms as melanomas, lymphomas [24], leukemias [27], and colonic carcinomas [28]. Functionally similar to NK cells, these T cells generated considerable confusion for several years in the characterization of the NK phenomenon. Though constituting the vast majority of "NK clones" successfully grown in culture [29], these T cells were inappropriately termed NK cells, as subsequent studies showed by revealing that the bulk of NK activity *in vivo* is mediated by cells markedly distinct from the T-cell lineage. These NK-like T cells are now termed "MHC non-restricted CTLs" and constitute <10 percent of the NK active cells *in vivo* [28]. Surface markers of these MHC non-restricted CTLs are similar to those described for MHC-restricted CTL, including CD3 and, often, CD8. In addition, these cells also display NKH-1 (also known as Leu-19), a marker present on the majority of NK cells [28].

Recent work has suggested that this newly characterized group of CTLs may be further subdivided on the basis of the recognition mechanism. There is evidence for two subgroups of MHC non-restricted CTLs that employ a CD3-complexed T-cell receptor structure: one expresses CD8 and appears to use the classical α-β heterodimer for antigen recognition [27], and the other is CD3+,CD8($-$) and appears to employ the alternative γ-δ or γ-γ dimer [16]. In both groups, the TcR is utilized without clear involvement of CD8 or requirement for target cell MHC recognition. Recently there has been support for recognition via a non-TcR mechanism as well as in certain MHC non-restricted CTL clones, with the exact nature of such recognition currently undefined [56]. As in MHC-restricted CTLs, lytic mechanisms used by MHC non-restricted CTLs include PFPs and soluble mediators [22] and activators of cytolysis include IL-2 [24]. The ontogeny of MHC non-restricted CTLs has yet to be studied exhaustively but is presently indistinguishable from that of the majority of T cells. MHC non-restricted T cells, then, are a distinct population of host effectors active against a wide variety of neoplastic cells and only recently differentiated in the literature from NK cells. How these cells become MHC non-restricted and possible roles for them in immunotherapy of neoplasia is a matter of current investigation.

NATURAL KILLER CELLS

The NK cell is the classical cellular mediator of the innate, nonspecific branch of the immune system, in that its recognition is MHC non-restricted and includes a broad

rather than a specific target repertoire independent of the need for prior exposure to antigen for priming and generation of immunologic memory. Previously characterized as a phenomenon believed mediated by a vast and heterogeneous variety of cell types, recent work in molecular biology and cell surface phenotyping has identified the mediators of the bulk of natural killer activity *in vivo* as a specific cell type, the NK cell. In addition to lysing neoplastic cells, NK cells function in host resistance to viral infection [30], radioresistant rejection of transplanted tissue grafts, immunoregulation, and regulation of normal hematopoietic populations. These other roles for the NK cell in immunoregulation as well as regulation of hematopoietic cell development have received increasing support. For example, NK cells secrete a host of lymphokines, including IL-2, IFN, and BSF-1, and have been shown to modulate immune effectors *in vitro* by suppressing dendritic cell function in culture with subsequent suppression of CTL function [31]. NK cells have also been shown to suppress T-cell response in autologous mixed lymphocyte culture systems [32]. Regulation of normal populations has also been suggested, because NK cells can kill immature cells in hematopoietic populations as a function of their differentiation state [33].

Activity against neoplastic cells is the best studied of all NK functions. Originally defined as the ability to lyse murine YAC-1 and human K562 *in vitro,* this activity has been identified against many other targets, as well as shown to be active *in vivo* by both depletion and augmentation studies. *In vitro* studies have demonstrated NK activity against a variety of neoplastic lines, with many transformed populations being resistant [34]. Spontaneous lysis of fresh and cultured tumor cells has been repeatedly demonstrated by washed/serum-free large granular lymphocytes (LGL) of human PBL and mouse spleen in four- to eight-hour incubation chromium release assays. Since the first *in vitro* studies of NK function in the 1970s, a number of experiments have supported the role of NK cells in resistance to neoplasia *in vivo.* Mice treated with the anti-NK serum AsGM1 have clearly increased numbers of metastases in experimental tumor systems [35]. A number of investigators have also correlated an individual animal's NK activity with its susceptibility to experimentally induced neoplastic cells [36–40]. Human support for this model includes the finding that patients with decreased NK function found in the Chediak-Higashi syndrome have a markedly increased evidence of lymphoproliferative disorders. All this evidence points to a significant role for NK effectors in host defense against neoplasia.

NK Cell Phenotype

Numerous previous studies in the literature have recently led to the definition of a well-defined series of cell markers that constitute the NK cell phenotype [41,42] and are listed below.

NK1.1, AsGM1 and 2, Ly5, Qa-2, Qa-5, NK2.1, Fc receptor	Murine
CD16, CD2, CD7, CD11, NKH-1, M1, Leu-7	Human

Cells expressing these molecules have been shown to be, aside from the small population of MHC non-restricted CTLs, the sole mediators of NK function *in vivo.* Like CTLs, NK cells use both PFPs [43] and soluble mediators [44] to lyse neoplastic cells and can be activated to do this with IL-2 and γ interferon [45].

NK Cell Recognition

How do NK cells recognize their targets? This is one of the currently unanswered questions in immunology and may hold some of the key insights into what makes a

neoplastic or virally infected cell identifiable by the immune system. The LGL population appears to be able spontaneously to "see" a neoplastic or virally infected cell and lyse it without previous exposure to that cell's surface determinants. Such "rough-and-ready" capacity for a wide variety of targets implies either a widely distributed recognition mechanism of remarkably broad specificity on all the 10–15 percent of peripheral blood lymphocytes with the NK phenotype, or it suggests a myriad of preprogrammed, highly specific mechanisms on many small subgroups of the LGL population. The evidence is in favor of the former. Very small numbers of freshly purified NK cells can lyse target cells in effector-target ratios as small as 1:1, indicating that at least a significant minority of NK cells can exercise the role of the whole population.

What, then, are possible recognition mechanisms for the NK cell? One possibility that has been favored in the past is the TcR. Largely secondary to the confusion resulting from generation of NK clones that are now being recognized as MHC unrestricted CTLs, the conventional 90,000 molecular-weight heterodimer, consisting of α and β chain subunits, was considered a viable candidate [29]. Recent studies indicating that freshly isolated NK cells do not rearrange their TcR α, β, and γ chain genes and do not express CD3 have effectively ruled out the conventional [28] as well as the alternative TcR. If not an alternative TcR, the other option allowing specific recognition of some broadly shared target characteristics involves a theoretical "NK receptor." This NK receptor would have an extremely broad repertoire, allowing it to recognize neoplastic, virally infected, and certain immature cell populations. It would perhaps have limited diversity and be linked to a transmembrane activation mediator analogous to CD3, such as CD2. No investigation has ever identified such a receptor. Some have suggested that NK activity is not mediated by specific recognition structures at all but occurs through entirely nonspecific mechanisms. For example, total changes in target cell MHC expression or modification of such surface components as the glycocalyx, ubiquitin content, or transferrin receptors represents nonspecific quantitative changes in the target cell that could be recognized merely by something like increased adhesion by NK cells.

Antibody-dependent cell-mediated cytotoxicity may be another possibility *in vivo* that has been overlooked. NK cells express FcR for IgG. Specific antibodies to tumor-associated antigens or viruses bound tightly by these FcR may serve as the specific recognition structure for NK cells, but definitive proof for this hypothesis is lacking. The means by which NK cells recognize their targets, then, remains a mystery. Specific receptors have not been identified, and this question continues to be a major avenue for future investigation.

Ontogeny of NK Cells

The ontogeny of NK cells has been a matter of considerable debate recently, but the evidence has been steadily accumulating to suggest that these cells constitute a distinct leukocyte lineage. Early arguments favored the belief that NK cells were merely immature T cells that never "grew up," largely based on their expression, like Stage II thymocytes, of CD2 and the fact that athymic animals often exhibited increased levels of NK activity. Evidence resulting from recent work has failed to support this possibility. Other cells, such as macrophages, share common T and NK markers like CD2, and there is a total lack of evidence that NK cells can be induced to differentiate into T cells. Furthermore, NK cells, unlike Stage I thymocytes, do not rearrange their

γ chain genes of the TcR [46]. Progenitor studies have also been convincing. W/W^v mice have an intrinsic deficiency of myeloid stem cells while severe combined immunodeficiency (scid)-diseased mice have a defect in early T- and B-cell differentiation [47]. Yet both mice strains have normal amounts of NK precursors capable of differentiation into mature NK cells [48].

Other attempts to define ontogeny of NK cells have proposed that they are members of the myeloid lineage, primarily based on their expression of FcR and M1. M1, however, is now known to be the complement receptor for C3b and is found on many cell types. Also arguing against this possibility is the fact that many antigens found on NK cells, including CD2, CD1, and Leu-7, are not found in the conventional myeloid series. At present, the best evidence seems to argue that NK cells may represent a distinct leukocyte lineage with properties of both lymphoid and myeloid population.

NK cells, then, constitute a distinct class of immunologic effectors active in host destruction of neoplastic cells. The recognition mechanism they employ in detecting neoplastic cells is currently a major unanswered question in immunology.

LAK PHENOMENON

Lymphokine-activated killers (LAKs) are unfractionated leukocytes, incubated in tissue culture with IL-2, which exert potent cytolytic activity against a wide range of neoplastic cells *in vitro* [49,50]. Initially hailed as a distinct lineage of immunologic effector [50] and potent immunotherapy for neoplasia [51], the "LAK" phenomenon has proved to be neither [52,58,2]. The effectors mediating this phenomenon appear to be derived over 90 percent from activated NK cells, with the remainder of activity mediated by activated MHC non-restricted CTLs [41]. One physiologic insight this phenomenon may provide, however, is an analogy to the expansion in the target repertoire and lytic activity possible in NK cells exposed to lymphokines released in a local area of tumor involvement.

NATURAL CELL-MEDIATED CYTOTOXICITY (NCMC) POPULATION

NCMC is a recently identified phenomenon mediated by large granular lymphocytes with lytic activity against human sarcomas *in vitro* [53]. Phenotypically these cells express HNC-1A3, M1, Leu-11 antigens in humans, with the precise nature of T-cell surface antigen expression a matter of current study [53,54]. The classical NCMC neoplastic target *in vitro* is the human fibrosarcoma WEHI-164. The mechanism of target cell recognition is unclear, but the lytic process has been shown to be mediated by the soluble mediator tumor necrosis factor (TNF) [55]. Activation requirements and kinetics of cytotoxicity are markedly different from NK cells in that IL-3, not IL-2, stimulates cytolysis and that peak target lysis is achieved in the 12- to 18-hour assays, as opposed to four to eight hours for NK cells. The ontogeny of the NCMC population is currently not well defined, and the overall contribution of this set of host effectors in defense neoplasia remains to be studied.

SUMMARY

Characteristics of the cytolytic cells active against neoplasia, then, have been identified in such a manner as to allow their distinct categorization (summarized in Table 3). Much more work in this area is needed to understand the recognition processes used, and to allow manipulation of the immune response to neoplastic cells. Initial trials of cellular immunotherapy of neoplasia in humans have been plagued by

TABLE 3

Cytolytic Effector Cell Types Active Against Neoplasia

	MHC-Restricted CTLs	MHC Unrestricted CTLs	NK Cells	LAK Phenomenon	NCMC
Cell Markers	CD2, CD3, CD7, CD4 or CD8, CD5, TcR	As MHC-Restricted CTL (1) CD8, a/b* TcR (2) gamma** TcR	CD16, NKH-1 CD2, CD7, Leu7	As (1) CTL (2) NK	HNC-1A3 M1, Leu11 ? t Cell Ags
Recognition Structure	a/b TcR	(1) a/b TcR (2) gamma TcR (3) Unknown mechanism	Unknown	As (1) CTL (2) NK	Unknown
Target Repertoire					
K562	NT	+++	+++	+++	
Fresh solid tumors	+++	+	+	+++	NT
WEHI	NT	NT	—	NT	+++
Cytolytic Mechanisms	PFPs, SM***	PFPs, SM	PFPs, SM	PFPs, SM	SM
Activation Requirements	IL-2	IL-2	IFN, IL-2	IL-2, ?IFN	IL-3
Morphology	Variable	LGL'	LGL	LGL	LAGL#
Max. CTX in vitro	Variable	4–8 hours	4–8 hours	4–8 hours	12–18 hours
Origin/Ontogeny		Thymically derived T cells	Probable distinct lineage Identifiable marrow precursor	As (1) CTL (2) NK	Unknown

*a/b denotes the alpha-beta heterodimer of the T-cell receptor.

**gamma denotes products of the TcR gamma gene complex with or without delta chain products.

***SM, soluble mediators   NT, not tested

K562, human myeloid leukemia line   WEHI, human fibrosarcoma line

'LGL, large granular lymphocytes (12 to 15 microns in diameter)

#LAGL, large agranular lymphocytes

+, Sensitive to lysis

−, Resistant to lysis

variable tumor response rates and toxic side effects of lymphokine therapy with a "leaky capillary" syndrome leading to massive anasarca [52], but the trials have yielded some encouraging results [2,51]. The success of future efforts will depend on elucidation of the fundamental properties of the cellular immune response against neoplasia through characterization of molecular mechanisms, development of appropriate animal test systems, and further appropriate trials in humans.

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