Natural killer cells and Their Relationship to T-Cells: Hypothesis on the Role of T-Cell Receptor Gene Rearrangement on the Course of Adaptive Differentiation

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Natural killer cells were discovered about 13 years ago during studies of cell-mediated cytotoxicity against tumor cells (see Ref. 1 for review). Although investigators in this area of research expected to find specific cytotoxic activity of tumor-bearing individuals against autologous tumor cells or against allogeneic tumors of similar or the same histological type, appreciable cytotoxic activity was also observed with lymphocytes of normal individuals. It soon became clear that such cytotoxic activity was mediated by a particular subpopulation of effector cells, which have come to be known as NK cells. 2

NK cells may be defined as effector cells with spontaneous cytotoxic reactivity against a variety of target cells, particularly tumor cells, tumor cell lines, and a limited variety of normal cells (e.g., virus-infected fibroblasts and subpopulations of bone marrow cells and thymus cells). The characteristics of NK cells and their possible relationship to T-cells or macrophages have been studied extensively (see Ref. 2 for review). NK cells have been clearly distinguished from typical macrophages, being nonphagocytic and mostly nonadherent and lacking some cell surface markers which are characteristic of most if not all monocytes and macrophages. Similarly, NK cells have been distinguished from CTL since their cytotoxic reactivity has not been found to be restricted by the MHC, with some of the most sensitive targets for NK activity lacking detectable MHC antigens, and NK cells have been shown to lack cell surface markers which are characteristic of CTL (e.g., Lyt-2 in the mouse). One of the most consistent features of NK cells, which has permitted their purification and detailed comparison with other types of effector cells, is their close association with a morphological subpopulation of cells, the LGL (3).

Over the past several years, much of the attention devoted to NK cells has concerned their possible in vivo relevance, particularly with regard to their possible roles in host defense against tumor growth (4). Some evidence has been accumulated to indicate a possible role of NK cells in immunosurveillance against certain types of tumors. More extensive and convincing evidence has been accumulated to indicate an important role of NK cells in resistance to progressive growth and metastasis of tumors (see Refs. 5 and 6 for recent reviews of this information).

There has also been much recent interest in elucidating the mechanism of cytotoxicity by NK cells (see Refs. 7 and 8 for reviews). A potent cytolytic protein, termed cytolsin, has been isolated from the granules of LGL (9) and this granule cytolsin appears to be intimately involved in the mechanism of cytotoxicity by NK cells. In addition, NK cells have been shown to release a soluble NK cytotoxic factor which might be an alternative but less efficient mechanism of lysis (10).

One of the most controversial areas of research on NK cells has concerned the lineage of NK cells and their possible relationship to the T-cell or macrophage lineages. Three major alternatives have been extensively considered: (a) NK cells may be derived from, and closely associated with, the myelomonocytic lineage, and most closely related to macrophages; (b) NK cells may be related to the T-cell lineage; (c) NK cells may represent a separate lineage derived from bone marrow stem cells and they simply may share some cell surface and other characteristics with cells from other bone marrow-derived lineages.

Most of the experimental evidence for an association of NK cells with macrophages has been relatively weak and could be otherwise accounted for. The expression of OKM1 or Mac 1 on NK cells was initially taken as indicative of an association with macrophages (11, 12), but recent evidence has indicated that these antigens are on the cell surface receptor for C3bi and are not restricted to macrophages (13), being expressed also on a subset of T-cells which appear unrelated to NK cells. A study with mouse bone marrow cells indicated that NK activity developed from a population enriched in macrophage precursors (14). However, the population containing the NK precursors was not a pure population of macrophages and could have also contained nonmacrophage precursors for NK cells. Along the same lines, Roder et al. (15) reported that a population of cells highly enriched in NK activity could undergo an oxidative burst and this evidence was taken as definitive evidence for an association of NK cells with macrophages (16). However, subsequent studies indicated that the generation of the oxidative burst was dependent on the interaction of a small number of contaminating macrophages with the NK cell population (17). Another indication for the lack of an association of NK cells with macrophages has come from studies of the expression of receptors for colony-stimulating factor 1 which have been found to be expressed on most cells in the macrophage lineage. These receptors could not be detected on a purified population of rat NK cells.

The possible relationship of NK cells with the T-cell lineage has been considerably more difficult to evaluate. On the one hand, it has been clear that NK cells are different from mature or classical T-cells. High levels of NK activity have been found in nude athymic or neonatally thymectomized mice and rats (18, 19). Characteristic markers of T-cells, such as Lyt-2 for mouse cells (20) or T3 or TiOl for human cells (21), have been undetectable on NK cells. In addition, recent studies have indicated that there is a lack of a productive rearrangement of T-cell receptor genes in rat LGL leukemias (22) or in normal LGL of various species (23).

Despite such negative evidence, there have been numerous
suggestions for some relationship of NK cells to T-cells:

1. NK cells have been found to express some T-cell-associated markers. The majority of human NK cells express low-affinity receptors for sheep erythrocytes (24) and react with monoclonal antibodies against these receptors (21). A subset of human NK cells also expresses the T8 antigen, a characteristic marker for the cytotoxic-suppressor subset of human T-cells (21) and most rat NK cells express the analogous OX8 marker (25).

2. NK cells as well as T-cells respond to IL-2, with stimulation of cytotoxic activity and also proliferation (26–28). The IL-2-dependent growth of NK cells has been found to depend on expression of IL-2 receptors which are antigenically the same as on activated T-cells (28). However, stimulation of cytotoxicity of human NK cells appears to be independent of the Tac IL-2 receptor (28).

3. The majority of human Tγ leukemias have been found to have most of the characteristics of LGL, express characteristic T-cell markers, often have NK activity (29), and have rearrangements of the gene for the β-chain of the T-cell receptor (23).

4. NK cells and CTL appear to share similar if not identical lytic mechanisms. The characteristics of the cytolysin isolated from the granules of LGL (9) are virtually identical to those described for the perforin of CTL (30). In contrast, cytotoxic macrophages do not appear to have an analogous granule cytolysin.5

5. There have been recent indications for a sharing of the LGL morphology by T-cells in some situations. Biron et al. (71) have recently described virus-induced in vivo generation of alloreactive CTL with LGL morphology.

6. Highly purified populations of human LGL, devoid of detectable T-cells, have been found to produce several T-cell-associated cytokines, including γ-interferon and IL-2 (26, 31).

7. Highly purified populations of human LGL, initially depleted of any detectable T-cells, have been found to develop typical T-cell markers upon in vitro culture in the presence of IL-2 (32). At least some of the cultured cells with such T-cell markers have high levels of NK activity. Recently, it has been shown that in parallel with the acquisition of T3 by human T8+ LGL in culture, the cells also begin to show rearrangements of the β-chain of the T-cell receptor (23).

8. In addition, many situations have been described in which cultures of typical T-cells develop NK-like activity (33, 34). This has also been shown to occur with cloned CTL, which develop NK-like activity simultaneous with continued expression of typical MHC-restricted CTL activity (35, 36), or loss of CTL activity (37). Some of these T-cell clones also may express markers which have been closely associated with NK cells, e.g., NKH1 (35).

One group has suggested that the receptor for NK-like activity may be the T-cell receptor, since antibodies to idiotypic specificity on the cloned cells reacted with the typical heterodimer associated with the T-cell receptor, and this antibody blocked the NK-like cytotoxicity (35, 38). In contrast, however, another group, working with clones of rat CTL, showed that the NK-like activity appeared to be due to receptors distinct from the T-cell receptors for antigens (36). To reconcile this divergent finding, one might postulate that in the former studies (35, 38), blocking of NK-like recognition by anti-clonotypic antibodies was due to steric hindrance of NK receptors in close proximity to T-cell receptors.

Because of the large amount of experimental results which

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have been generated with regard to the possible relationship of NK cells and T-cells, which often seem rather contradictory or divergent, it seems quite important to reassess the data and to provide a theoretical framework to accommodate most if not all of the available information. As an early hypothesis on the association between NK cells and T-cells, it was suggested, based on the data showing high levels of NK activity in nude or neonatally thymectomized mice, that NK cells might represent a stage of prethymic T-cells, which accumulate when the ability to further differentiate into mature T-cells is blocked by the absence of the thymus (39). Another hypothesis, along the same line, suggested that NK cells represented an early stage in the development of T-cells and that both effector cell types shared similar receptors (40). Subsequently, Klein (41) noted that NK-like activity develops in alloantigen-stimulated cultures prior to the development of CTL activity and suggested that NK activity represents a polyclonal response of T-cells during early stages of activation and prior to the development of MHC-restricted reactivity to specific antigens. Several years ago, we also proposed a hypothesis on the origin and specificity of NK cells (42). This hypothesis offered an interpretation of the similarities and differences between NK cells and CTL and was based on the assumption of a common T-cell lineage but distinct specificity repertoires. CTL either recognize MHC antigens or recognize other antigens in association with MHC products, whereas NK cells interact with a different set of cell surface antigens. We argued that since precursor lymphocytes undergo dynamic selection (43, 44) which is driven by self-antigens that they recognize, the pattern and consequences of this selection must depend on the nature of the antigens recognized and on the nature of the target cells presenting these antigens. Several major properties are thus affected by this dynamic selection: (a) the identity of the targets for effector reactivity in relation to the reference self-antigens ("restriction"); (b) the degree of cross-reactivity among effector clones; and (c) the predominant states of maturation of the selected cells and the associated phenotypic and functional properties of the effector cells.

We now propose to reevaluate this hypothesis, based on the considerable accumulation of relevant data over the last 3 years, and to then extend or modify the hypothesis based on the recent data. In addition to the information discussed above, our revised hypothesis has been influenced by more detailed information on the characteristics of NK cells and LGL, their cell surface markers, and their responsiveness to various inducers; studies of cultured clones of LGL and T-cells; and in vivo kinetics of activation and proliferation under different conditions.

General Considerations

The definition of NK cells is largely related to their functional activities, i.e.: (a) their spontaneous or natural expression of effector activity and the lack of an apparent need for prior immunization or proliferation; and (b) their specificity repertoire or their pattern of cytotoxicity against certain target cells but not others. In addition, as discussed above, NK cells have been closely associated with characteristic morphological markers. Although such a definition has been very helpful for classifying these effector cells and distinguishing them from various other effector cells, there are several major problems with precisely defining NK cells, including the observations that some markers associated with NK cells are not expressed on all of these effector cells and further, most of the NK-associated markers are not unique to NK cells but also may be expressed on other cell types. For example, as noted above, some LGL...
are T-cells, including CTL. In addition, a definition linked directly to NK activity does not include the NK precursors, i.e., cells which do not have effector activity but which may require stimulation or differentiation to develop activity. These precursors of NK cells also may lack some of the characteristic markers of functional NK cells. It would seem most helpful for both theoretical and experimental purposes to include precursors of NK cells within the same considerations as functionally active NK cells. Thus, a somewhat more general and satisfactory definition might be "cells with the ability to selectively recognize a given range of (NK-susceptible) target cells, with the potential to lyse these cells, either immediately or upon further maturation or activation." Once sensitive and specific means to identify cells with NK recognition receptors (either by appropriate antibodies or by molecular biological approaches) are available, a more precise categorization of NK cells and their precursors might be possible. In the meanwhile, we can define NK cells and some of their precursors only by conjugate assays, which have the limitation that different effector cell types may bind to the same target cell by different types of receptors. In any event, our premise is that the NK recognition structure is the central issue regarding the definition of NK cells and that other characteristics, e.g., the markers or other aspects of phenotype, will be associated with such a definition but not completely coincident. We predict that these other attributes would be strongly influenced by the dynamic mechanisms which regulate the state of differentiation and maturation (42). We postulate that the interaction of the recognized structures on target cells, i.e., antigens, with the receptors on T-cells or NK cells plays a central role in regulating their growth and maturation.

According to our hypothesis, clones of lymphocytes and NK cells resemble competing species in a Darwinian sense (43). External antigens and self-antigens may stimulate several clones which differ in specificity, affinity, or class and, consequently, in their growth capacity. Each clone can in turn trigger negative feedback control, which may or may not affect other clones. Cross-reactive stimulation and control define a competitive relationship among these clones and can lead to suppression of some clones, depending on the particular pattern of cross-reactivity and on the duration and strength of stimulation. The outcome of this dynamic selection depends on the growth capacities of the competing clones and these are assessed by the "balance of growth" hypothesis (44). We propose that precursors of T-lymphocytes and NK cells possess a capacity for self-renewal and that antigen-activated, but not resting, cells can be further induced to mature or regenerate, and their relative probability of maturation increases with antigen dose or with affinity.

The further aspect of this hypothesis is that the in vivo induction of terminal differentiation of T-cells and NK cells is antagonistic to clonal expansion. The hypothesis leads, in particular, to the following interpretations of self-tolerance and restriction: (a) self-tolerance would be attributed to the impairment of growth of clones which recognize abundant self-antigens with high affinities. These clones are instead induced to differentiation and consequently they are eliminated through dynamic selection; (b) restriction would result from the proliferation of cells recognizing these self-antigens with a certain range of low affinities, and these thereby gain prominence in the dynamic selection. Recognition of nonself-antigens by the positively selected repertoire of clones must be associated with, or related to, recognition of self.

One may then ask which self-molecules can serve as restricting elements in the recognition of foreign antigens. We postulate that any self-antigen that actively participates in the generation of tolerance is automatically restricting; the clones which it positively selects will be able to respond only to similar antigens. The ability of a self-molecule to be a major restricting element depends on a number of factors: (a) its abundance; (b) its potential to associate with, or be initiated by, foreign antigens; and (c) the capacity of the antigen-positive target cell to induce significant proliferation in clones with low-affinity receptors. The MHC-coded molecules certainly have all these three characteristics and can account for the restriction associated with T-cells. Other cell surface molecules might serve this same function for NK cells.

We previously proposed (42) that NK cell precursors arise from the T-cell lineage and initially differ from T-cell precursors only in the expression of different sets of receptors. We postulated the existence of a class of molecules that serve as reference antigens in the development of NK clones and direct their effector activity. We further postulated that the interaction of relatively mature NK precursors with target cells expressing the appropriate molecules is a weak one in the sense that the relative probability of maturation versus self-renewal is inherently large. Therefore, except under special conditions (prevailing presumably in the environment of the bone marrow; see below), self reference antigens, and especially modified self-antigens, tend to drive NK cell precursors towards terminal differentiation, with little amplification. In contrast, recognition of MHC antigens mediates a series of events, which includes the involvement of growth factors, which often leads to extensive amplification in MHC-specific T-cells. The cytolytically active NK cell is postulated to be predominantly a terminally differentiated, nondividing cell. A recent experimental study has provided support for this hypothesis. Zagury et al. [45], using a single-cell micromanipulation technique, isolated cytolytic effector cells from clones of LGL or of CTL and placed them in culture with IL-2. Less than 10% of the NK effector cells were able to proliferate. The active CTL appeared to have even lower proliferative potential, with none of more than 150 cells showing evidence of proliferation.

Our hypothesis departed from other hypotheses that either regarded the NK lineage as completely unrelated to the T-cell lineage or identified the NK effector cells with an early, immature T-cell (e.g., Ref. 40) or with a form or stage of activation of a classical CTL (41). As noted above, there is experimental support for the concept that active NK cells are well differentiated with only limited proliferative potential. Along the same lines, Alleva et al. [46], using a limiting dilution assay, showed that the frequency of cytotoxic progenitors derived from LGL was only about 1 in 100 and that within this population the effector cells expressing the Fc receptor, as detected by the B73.1 monoclonal antibody, had a 5-fold lower frequency than the B73.1-negative LGL. Thus, it appears that most of the cell lines and clones displaying NK-like reactivity do not arise from mature NK cells but rather from precursors in the same population. Also, the demonstration that the development of NK-like activity in some CTL clones may be attributable to different sets of receptors, one for the MHC-restricted recognition of CTL-associated antigens and another for the NK cell targets (36), must be incorporated into any hypothesis about the relationship between NK cells and T-cells. Clearly such findings support our basic assumption of different receptor repertoires.

On the basis of the recent advances in our understanding of recognition by T-cells, we now propose that the point of departure between T-cells and NK cells during the pathway of differentiation in the T-cell lineage is when the T-cell receptor gene...
is rearranged and expressed. We suggest that the expression of receptors for NK cell targets is probably an earlier event in ontogeny. The nonrearranged lymphocytes continue to interact with their cellular environment, in part through these receptors. This leads to expansion and maturation, associated also with increased receptor expression. The capacity to express NK cell receptors declines in maturing T-cells along with the maturation of the MHC-restricted recognition mechanism but can be regenerated even late in maturation by appropriate and persistent external stimuli as may be frequently or even regularly observed in CTL clones under some environmental circumstances, particularly the presence of high concentrations of IL-2 (35). A schematic representation of our proposed model of NK cell and T-cell differentiation is depicted in Fig. 1.

The usual concept of lineages is based on the premise that, following some discrete genetic events of determination or commitment, immature precursor cells continue along an intrinsically programmed sequence of divisions and maturation steps toward a terminal state. In the lymphocytic lineages, it has been realized that the number of divisions in some intermediate states of differentiation is subject to external regulation, but it is still generally believed that the differentiation pathway of normal cells is fixed in advance, under all conditions. This provides the rationale for many in vitro experiments which aim to take a particular subpopulation of cells out of the complex physiological environment and place them into the simpler culture environment where they could be studied in detail. The assumption is that the events observed in vitro will accurately reflect, and provide insights into, the analogous sequence of events which occur during in vivo differentiation. This approach ignores the excessive phenotypic plasticity which characterizes cultured cells. For example, Zagury et al. (45) showed that culture of individual cells selected for a particular marker could result in the acquisition of a different set of markers. Such shifts have not as yet been observed to occur in vivo and thus appear to be rare or nonexistent. Similarly, the observed shifts in functional phenotypes between CTL and NK-like activities (33, 34) may not reflect usual in vivo lines of differentiation. Overall, the evidence indicates that a cell is not an independent entity in terms of phenotype or function but rather reflects both environmental stimuli and intrinsic properties.

We believe that this observed plasticity reflects the normal potential for phenotypic adaptation, namely the capacity of cells to change the pattern of gene expression in response to changes in the environment. Tissue interactions determine the developmental fate of cells in the embryo and are required even in adult life to maintain the identity of cells (47). A role for DNA methylation in stabilizing epigenetically induced changes in gene expression has been proposed (48, 49). In this regard, it will be of considerable interest to carefully examine and compare the patterns of methylation of the DNA in NK cells and in T-cells.

The orderly, predictable pattern of differentiation in vivo, like the sequence of developmental events in embryogenesis, owes its regularity to a network of causal relations and not just to the unfolding of a genetic program (50). Following receptor gene rearrangement and expression, T-cells are subject to different regulatory or inductive signals as compared to nonrearranged cells, and the differentiation pathways of these two sets of cells necessarily diverge from each other. Thus, the development of each set of cells will be associated with those organs and tissues which favor proliferation of a particular cell type. This in turn leads to an association of each population with additional microenvironmental factors which can affect the growth characteristics and the phenotype.

In our previous communication (42), we emphasized the consequences of the “weak interaction” conjecture with regard to the morphological and functional characteristics of the NK subpopulation. The essence of this conjecture is that NK cell precursors are driven more readily to maturation as a result of interactions with the self-environment and particularly when

![Schematic model of NK cell and T-cell differentiation](image-url)
stimulated by a relevant external challenge. Consequently, we identified the LGL phenotype and morphology with a close to terminal stage of differentiation in the NK-T-cell lineage. We therefore predicted that phenotypic switches from CTL to the LGL phenotype might frequently occur, in vitro and in vivo. At that time, such switches had not been observed. However, since then, such shifts in phenotype have been observed in culture (34, 51) and recently the LGL phenotype has been shown to be a characteristic which may be common to both NK cells and activated functional CTL in vivo (71). The mature character of functionally active NK cells would suggest that they would be short-lived. Indeed, recent data have indicated that LGL have half-lives of less than 5 days (52). Along with this, one might expect to have an extensively proliferating precursor population at some earlier stage of differentiation, and indeed a high level of such proliferative activity has been observed (53). In our original hypothesis, these kinetic characteristics were postulated to explain the natural reactivity of NK cells, as contrasted to the requirement for priming and memory in CTL-mediated immune responses.

In summary, our hypothesis predicts distinct morphological, functional, and distribution characteristics, and also more subtle differences in the pattern of gene expression, for NK cells and T-cells. Such observed differences (e.g., Ref. 54) may be causally related to the T-cell receptor rearrangement and expression in a portion of the common precursors but not in others. We postulate that the differences develop dynamically and are subject to mutable environmental factors. In a sense, the rearrangement of the T-cell receptor gene can be seen as a commitment event, in that it endows the cells with a characteristic that substantially affects their future growth and maturation. However, assignment of the NK cell or LGL to some elusive alternative lineage is unnecessary. Finally, we postulate that in culture T-cells and NK cell precursors express variable patterns of gene expression. This makes them an interesting subject for studies of adaptive differentiation but this also limits our ability to make inferences from such studies about cell characteristics and in vivo regulatory pathways.

Speculations on Selective Recognition and on Regulation

A. Discrimination between Self and Nonselby NK Cells. According to the above hypothesis, the existing repertoire of NK cells results from a potentially larger repertoire by a process of competition and selection. Cells are selected which respond by extensive proliferation to stimulation by certain types of autologous cells, during some phase of their development. The strength of interaction (affinity) should be sufficiently small to avoid premature terminal differentiation and this constraint ensures self-tolerance. Foreign antigens are recognized as "modified self" with different, often higher, affinities to the extent that they resemble the original self-antigens or are associated with them to form modified structures. This may explain the observation that NK cells are generally more reactive against transformed cells. This would also provide an explanation for the observation that NK-resistant normal cells become susceptible to NK activity after being infected with various viruses or other microorganisms (e.g., Refs. 55 and 56). Further kinetic and molecular analyses are required to prove that such changes are due to more efficient binding and to identify the nature of the modified molecules. Self-nonsel discrimination is the most basic specificity characteristic of NK cells, an attribute shared with classical T-cells and B-cells and probably also by other types of cells such as macrophages, the effector function of which is usually not considered to be immunologically specific. The very process of adaptation to the self microenvironment may endow these effector cell populations with the capacity of surveillance against structural aberrations.

The specificity of MHC-restricted T-cell recognition is generally believed to stem from clonal distribution of receptors on the T-cells and the polymorphism of the targets. NK cells and their targets may differ in both respects, in that NK cells may bear multiple receptors and target cells may share a small number of recognizable structures which are broadly distributed. This may account, at least in part, for the observation that NK cells from different sources, including clones, show similar specificity patterns on panels of target cells (57). These patterns, however, are not identical and can be shown to vary among subpopulations and clones of NK cells. The concept of multiple receptors on NK cells, each for recognition of somewhat different specificities, is supported both by cold target inhibition experiments (58) and by the loss of reactivity of cloned populations against some NK-susceptible targets but not against others (59). However, in addition to such observed shifts in specificity, there appear to be some consistent differences in specificity among clones, supporting the possibility that some recognition structures on NK cells are clonally distributed, despite considerable overlap among receptor sets, or cross-reactivity of target antigens, as indicated by the lack of fine specificity.

We have previously argued (42) that the thymus is not an appropriate site for selection for NK cells, due to the strong competition for T-cell-directed differentiation in this organ. Rather, the bone marrow and perhaps the spleen are likely sites for differentiation along the NK cell pathway. Recent studies have confirmed a rapid renewal of NK cells in the bone marrow (e.g., Ref. 60). As demonstrated earlier for T-cells, it is predicted that NK cell precursors may be reeducated, if the microenvironment in which the NK cell repertoire is selected is antigenically modified, with resultant changes in the pattern of specificity of the NK cells which develop. The ability of some NK cells to react with hematopoietic stem cells in the bone marrow appears to offer a good system for analysis of this aspect of the hypothesis. Recent evidence indicates that NK cells may have the ability to recognize hematopoietic histocompatibility antigens and to discriminate parental bone marrow cells from those of hybrids (61). In vivo experiments previously demonstrated that neonatal injection of parental bone marrow cells into hybrid mice induced tolerance to subsequent transplantation of parental cells (62). Such observations are quite compatible with our present hypothesis.

B. Recognition of Self and Growth Regulation. Not only foreign structures but also autologous cell surface molecules may appear as modified self. The modification may be manifested as changes in the structure, pattern, or density of molecules on the cell membranes, associated with transformation or with normal maturational processes. For example, one might envision that NK cells are adapted to tolerate bone marrow hematopoietic cells over some range of maturational states but retain the ability to interact with and inhibit the growth of autologous hematopoietic cells which lie outside this range. This might explain the observations of reactivity of NK cells against some autologous or syngeneic bone marrow cells (63, 64) and also the NK cell-associated rapid in vivo elimination of some autologous bone marrow cells (65). It will be of considerable interest to further explore the ability to selectively educate NK cells and to modify their pattern of specificity, utilizing...
more highly selected target cell populations and highly selected populations of NK cells for interaction with these targets.

The NK resistance of some tumor cells might be interpreted as a form of tolerance, resulting from coadaptation of NK cell precursors and tumor cells. This is particularly conceivable for leukemias of bone marrow origin. In contrast, NK cells might interact more effectively with phenotypically modified tumors originating in organs and tissues that are not associated with NK cell development.

Another potentially important mechanism for regulation of differentiation of NK cells may be feedback growth control by the progeny of proliferating cells. For example, interferon, which can be secreted by mature NK cells as well as by T-cells (66), may regulate both the expression of effector reactivity and the growth of NK cell precursors. Evidence gathered in the last few years indicates that in vivo administration of interferon or a variety of other immunomodulators results in a substantial increase in the number of morphologically identifiable LGL in the spleen, liver, and other organs, and this is accompanied by an increase in the proportion of cells undergoing DNA synthesis (67–69). These observations could be attributed to the induction of terminal differentiation of mature NK cells from their precursors, which in turn accelerates the turnover in the tissues and triggers, through feedback interactions, a proliferative response in the bone marrow or other precursor compartments.

Other factors and cells are very likely to be involved in the regulation of differentiation of NK cells. In analogy to helper T (Th)-cells and suppressor T (T.)-cells as populations of T-cells specializing in regulation of T-cell functions, one might predict the existence of natural helper (Nh) cells and natural suppressor (Ns) cells within the NK cell population. These cells would share the same set of receptors with NK active cells and might have the associated phenotypic and functional characteristics but also the capability to produce lymphokines. Indeed, LGL with no apparent cytotoxicity but secreting growth and/or differentiation factors (e.g., IL-2, interferon) have been identified (70).

Our proposed model for NK cell and T-cell differentiation (Fig. 1) should generate a series of testable predictions. For example, an event that leads to expression of the NK cell receptor during an early phase of differentiation of NK cells and T-cells might be identified in the precursors of both. Our hypothesis predicts that NK cell precursors should have the capability under some circumstances to be induced to develop along the classical T-cell pathway. Certainly, hints of this switch have come from studies of cultured LGL, in which some cells appear to undergo rearrangements of the gene for the β-chain of the T-cell receptor and develop T-cell-associated markers (23, 32, 45). Such changes in phenotype need to be defined more precisely and, in particular, it will be important to determine whether cells with NK receptors but without T-cell markers can be induced to develop the phenotype and, of most interest, the characteristic functions of T-cells.

Another prediction from our model is that cells undergoing rearrangement of the gene for the T-cell receptor would be found to show a progressive decrease in expression of NK receptors. Clones of cultured LGL might provide a useful model to examine this prediction. Clones could be selected on the basis of whether they had undergone rearrangement of the T-cell receptor genes and then followed to determine the time course of retention of NK recognition structures. Our hypothesis predicts that the clones with rearrangement would show a greater decrease in NK receptors than the clones without rearranged T-cell receptor genes.

Summary and Conclusions

We propose that the differentiation of NK cells and the differentiation of T-cells are intimately interrelated, although mature effector cells of each type usually can be distinguished from each other. The divergence in their characteristics may be initiated upon rearrangement of the genes for the T-cell receptor, with a subsequent inverse relationship between the expression of T-cell receptors and NK cell receptors. However, an essential element of our hypothesis is that the differentiation of these cells is partially adaptive rather than rigidly preprogrammed. This concept is considerably more compatible with the phenotypic plasticity which has been exhibited by cultured cells in general and by T-cells and LGL in particular. We suggest that the nature of the self environment has a major influence on the direction of development of precursor cells, both by controlling the ratio between the rates of proliferation and differentiation at each stage of maturation and by inducing quantitative or qualitative changes in the pattern of gene expression. As maturation proceeds, the degree of plasticity probably decreases, possibly due to inheritable epigenetic changes in the genome. Our hypothesis accommodates most if not all of the available experimental data on the phenotypic, genetic, and functional interrelationships between NK cells and T-cells. In particular, it accounts for the extensive and controversial data on cultured cell lines with varying degrees of similarity to T-cells and to NK cells. In addition, our model emphasizes the inherent limitations in utilizing such data from cell lines as the basis for drawing conclusions on the properties of cells developing under physiological conditions. Most importantly, our hypothesis leads to a series of experimentally testable predictions, which should provide considerably greater insight into the ontogeny of NK cells and their relationship to the T-cell lineage.

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