The Immunophenotypic Attributes of NK Cells and NK-Cell Lineage Lymphoproliferative Disorders

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Abstract

Natural killer (NK) cells are a distinct lymphocyte subset that, like cytotoxic T cells, recognize and destroy abnormal self-cells. Unlike T cells, however, NK cells lack a uniquely rearranged antigen receptor gene or a single defining immunophenotypic attribute. Furthermore, there is considerable functional and phenotypic overlap between NK cells and cytotoxic T cells, and the latter are induced by cellular activation to express many NK-associated antigens. These factors all contribute to difficulties in recognizing abnormal NK-cell expansions and distinguishing them from cytotoxic T cells in the clinical laboratory. Recently, new classes of NK-associated major histocompatibility complex receptors have been described that can be of value in evaluating NK-cell and cytotoxic T-cell populations. This review, immunophenotyping approaches using antibodies to traditional and novel T- and NK-associated antigens that can be used to evaluate these cell types and diagnose NK-cell and cytotoxic T-cell disorders in the clinical laboratory setting are discussed.

Lymphocytes that exhibit major histocompatibility complex (MHC)-unrestricted target cell lysis in the absence of prior antigen exposure were first described in studies of tumor immunity and transplant rejection. Because this activity did not require antigen priming, it was termed natural cytotoxicity and was ascribed to natural killer (NK) cells. It was unclear, however, whether these functionally defined NK cells represented a true lymphocyte subset or rather a group of different lymphocyte types with common effector activity. Consensus was eventually reached regarding the defining features of a true NK cell type, and these included large granular lymphocyte cytology, the absence of T-cell receptor (TCR) gene rearrangements (ie, TCR genes in germline configuration) or a fully assembled TCR-CD3 complex, and the expression CD16 and CD56.

This definition reveals that NK cells, unlike B and T cells, lack a single unique genetic or phenotypic attribute but rather are identified by an amalgam of features both present and absent. Furthermore, NK cells and cytotoxic T cells are closely related in their ontogeny and function. This close relationship is reflected in their overlapping immunophenotype, as cytotoxic T cells often express NK cell–associated antigens such as CD56 and CD57, and NK cells normally express T cell–associated antigens such as CD2, CD7, and CD8. These similarities can make it difficult to distinguish these cell types in the clinical laboratory as illustrated by consideration of the TCR-CD3 complex. NK cells are CD3– by flow cytometric immunophenotyping (FCIP) because the anti-CD3 antibodies used in this assay recognize the fully assembled complex that NK cells lack. NK cells express some subunits of the CD3 complex, however, including the ε and ζ chains (Figure 1). This results in the reactivity of NK cells...
for CD3 in paraffin-section immunohistochemical analysis because this typically uses antibodies reactive with the CD3ε chain. Given issues such as these, the accurate immunophenotyping of NK cells requires a comprehensive approach using antibodies to a number of T and NK cell–associated antigens.

Traditional NK-Cell–Associated Antigens and Their Role in Diagnosing NK-Cell Proliferative Disorders

CD16, a receptor for the Fc portion of IgG (FcγRIII), was one of the first described NK cell–associated antigens; it enables cells to engage in antibody-dependent cellular cytotoxicity. CD16 occurs in 2 isoforms: CD16A, an integral membrane found on NK cells, and CD16B, a glycosyl phosphatidylinositol–linked protein expressed by granulocytes. Some anti-CD16 antibodies are isoform-specific, whereas others recognize both isoforms. When immunophenotyping NK cells, the use of a CD16A-specific antibody is preferable. Mature NK cells are virtually all CD16+, and they can be subdivided into 2 distinct subsets. One subset has bright CD16 and dim CD56 expression (approximately 90% of NK cells), and the other has dim surface CD16 and bright surface CD56 expression (remaining 10%). As with most if not all NK-cell antigens, CD16 is also present on a small proportion (<5%) of normal cytotoxic T cells. There are no currently available antibodies to CD16 that are reactive in paraffin-embedded material.

CD56, a neural cell adhesion molecule, is another early described NK cell–associated antigen. Although CD56 is commonly associated with NK-cell lineage, it is expressed on a wide variety of hematopoietic and nonhematopoietic cells, including T cells. Like CD16, CD56 expression by T cells is induced by cellular activation, although CD56+ T-cell populations are much more prevalent and are usually present even in normal peripheral blood specimens.

CD57 is a cell-surface glycoprotein that is expressed by approximately two thirds of NK cells and was first identified in this cell type. CD57 is also expressed by a number of other cell types, including memory cytotoxic T cells. The frequent expression of CD57 on T cells makes this antigen of relatively little value in distinguishing them from NK cells. As mentioned later, it may have some value in defining the aberrant immunophenotype of clonally expanded NK-cell populations.

Because NK cells lack a uniquely rearranged antigen receptor gene that can be used for clonality assessment, establishing the presence of an immunophenotypically distinct cell population is a central element in diagnosing NK-cell lymphoproliferative disorders. In paraffin-embedded material, NK-cell lymphomas are typically identified by the expression of CD2, CD56, and cytotoxic granule proteins such as granzyme B, as well as the absence of detectable TCRβ chain expression (using the β F1 antibody) or TCR gene rearrangements. As noted, NK cells can be CD3+ in paraffin-embedded material, and it should be considered that this immunophenotype does not exclude the possibility of γδ T-cell lineage with false-negative TCR gene rearrangement results. In peripheral blood and
bone marrow aspirate specimens, the consideration of an NK-cell disorder is usually triggered by the presence of increased large granular lymphocytes, often with associated cytopenias. Given that large granular lymphocytes can be cytotoxic T cells or NK cells, immunophenotyping approaches used in this setting should include antibody combinations that are useful in discriminating these cell types.

By FCIP, NK cells are typically identified by using antibodies to CD3 combined with anti-CD16 antibodies or an anti-CD16/anti-CD56 antibody cocktail in which both are labeled with the same fluorochrome. In these combinations, NK cells are CD3–/CD16+ or CD16+/CD56+. The rationale for the use of a CD16/CD56 antibody cocktail is that it detects the CD16 bright/CD56 dim and CD16 dim/CD56 bright NK-cell subsets. This cocktail reagent has drawbacks, however, because it does not allow for the distinction of these subsets or the identification of specific patterns of CD16 expression that can be diagnostically useful. Furthermore, because CD56 is often expressed by a significant proportion of normal T cells, this reagent has low specificity for identifying abnormal T-cell expansions with coexpression of NK cell–associated antigens, such as seen in T-cell large granular lymphocytic leukemia (T-LGL).14 Practically speaking, a single labeled anti-CD16 reagent can be used because most mature NK cells are CD16+ and increased numbers of CD3+ T-cells with coexpression of CD16 have greater specificity for a diagnosis of T-LGL.

The pattern of CD16 expression by NK cells can be useful in identifying abnormal NK-cell populations. In normal NK-cell populations, a range of CD16 expression should be observed. In NK-cell large granular lymphocytic leukemia (NK-LGL), the cells often exhibit uniform bright CD16 expression (Image 1A).14 Although this finding in isolation may not be sufficient to define NK cells as aberrant, when screening for such cells it can help earmark cases that require further examination. It is interesting that this bright expression of CD16 in NK-LGL tends to be associated with diminished to absent staining for CD56 and CD57 (Image 1B).

FCIP studies using antibodies to other T and NK cell–associated antigens can be helpful in characterizing NK-cell populations. As with CD3 and CD16, combinations of antibodies to T cell–specific and shared T- and NK-cell antigens, such as CD2 combined with CD5 and CD3 combined with CD7, are most efficacious. With this approach, normal NK cells are CD2+/CD5− and CD7+/CD3−. Direct comparison of the corresponding regions of these histograms can confirm an increase in NK cells and may reveal NK-cell populations that have aberrantly diminished expression of CD2 or CD7 and abnormal gain of expression of CD5 (Image 1A). This comparison is also helpful in detecting CD3+ T-cell populations with aberrantly diminished or absent expression of CD5, a feature often seen in T-LGL.14,15

Including antibodies to CD8 in combinations that allow for specific analysis of this antigen on NK cells is also of value. Normal NK cells typically show partial coexpression of CD8 (Figure 1), with a staining intensity that is approximately half that seen on the CD8+ T cells.16 In NK-cell lymphoproliferative disorders, the cells may be uniformly CD8+, a finding that is sufficiently distinctive to be considered aberrant (Image 1A). Given that normal NK cells are weakly CD8+, the absence of this antigen on NK cells is not sufficient to reliably distinguish them as abnormal.

FCIP analysis using antibodies to traditional T and NK cell–associated antigens is an important element in detecting abnormal NK-cell populations. However, the usefulness of such analyses is somewhat limited because abnormal NK cells can show normal patterns of expression of CD2, CD7, CD16, and CD56, and, conversely, normal NK cells can show altered expression of these antigens.17 The recently described NK-cell receptors (NKRs) provide a powerful tool for NK-cell analysis.

**NK-Cell–Associated MHC-I Receptors and Their Role in Diagnosing NK-Cell Lymphoproliferative Disorders**

Although NK cells, unlike T cells, do not recognize MHC-bound antigenic peptides, seminal observations revealed that the presence of self-MHC-I on potential target cells protected them from NK cell–mediated lysis.18 This protective effect was postulated to be mediated by “killing inhibitory receptors” on NK cells. These receptors are now known to be encoded by at least 2 distinct families of genes and gene products, including the killer cell immunoglobulin receptors (KIRs), which are members of the immunoglobulin gene superfamily, and the CD94/NKG2 complexes, which are members of the C-type lectin superfamily.19 These receptors have been recognized to occur in inhibitory and activating forms, and they are now broadly referred to as NK-cell receptors or NKRs, although these receptors are also expressed by a subset of memory cytotoxic T cells.20

Detailed discussion of this elegant group of receptors is beyond the scope of this article and has been recently reviewed elsewhere.10 Yet there are differences between these receptor types that bear directly on how they may be applied to NK-cell immunophenotyping. There are multiple KIR isoforms, each of which is encoded by a single gene and contains 2 or 3 extracellular immunoglobulin-like domains. These structurally distinct KIRs each recognize a specific group of MHC-I alleles. In contrast, the ligands for the CD94/NKG2 complexes are a group of MHC-I–related antigens with limited polymorphism such as HLA-E.21 From a diagnostic perspective the KIRs are of particular interest because they are stably expressed over multiple cell generations and, therefore, can serve as a marker of clonal cellular expansion. In contrast, the CD94/NKG2 complexes are not clonally expressed; however, it is possible to distinguish between the activating and
An aberrant natural killer (NK)-cell immunophenotype (arrows) in a case of NK-cell large granular lymphocytic leukemia (LGL). **A**, Flow cytometric immunophenotyping (FCIP) reveals the presence of increased CD3–, brightly CD16+ NK cells (upper). These cells are CD2+ and CD5−, a normal pattern of expression of these antigens in NK cells (lower left). The NK cells show abnormally diminished to absent expression of CD7 (lower middle) and abnormal, uniform expression of CD8 (lower right). **B**, **C**, and **D**, Additional multicolor FCIP allowed for specific gating on the CD16+/CD3− NK cells (upper, gate P2, arrow). By this technique, these cells have aberrantly diminished to absent staining for CD56 and dim expression of CD161 (**B**). The NK cells show restricted expression of the killer cell immunoglobulin receptor (KIR) CD158a. These cells lacked expression of CD158b and CD158e and the KIR NKAT2 (**C**, CD158e and NKAT2 data not shown).
inhibitory forms of the receptor complex with antibody reagents based on the identity of the NKG2 pairing partner.

The usefulness of KIR expression patterns in detecting clonal cellular expansions has been established, in large part, through FCIP studies of T-LGL. In this disorder of memory T cells, the uniform expression of a single (or multiple) KIR isoform has been found to be highly correlated with the presence of clonal TCR gene rearrangements.14,22 These observations have been validated by a number of other studies in T-LGL and other cytotoxic T-cell disorders using FCIP, frozen section immunohistochemical analysis, and molecular genetic approaches.15,23,24 In all of these studies, restricted KIR expression has been associated with the clonal cellular expansion. Paraffin-reactive antibody reagents to the KIR antigens are not currently available.

Despite the fact that NKRs are more uniformly expressed by NK cells than by T cells, there have been few studies of NKR expression in NK-LGL. These abnormalities of NKR expression in NK-LGL are associated with other features strongly correlated with clonality in T-LGL, such as the presence of intrasinusoidal bone marrow infiltrates.14 Restricted KIR expression has also been demonstrated in NK-cell lymphomas of the nasal type by reverse transcriptase–polymerase chain reaction and frozen section immunohistochemical methods.24,27

Unlike the KIRs, CD94/NKG2 heterodimers and similar receptors such as CD161 cannot serve as surrogate markers of clonality. Nevertheless, these antigens are still helpful in evaluating NK-cell populations. In NK-LGL, the abnormal cells often show uniform bright expression of CD94 exclusively paired with NKG2A to form an inhibitory receptor complex Image 1D.14,28 This is in contrast to normal NK cells in which there is variable staining intensity for CD94 with a mixture of CD94/NKG2A and CD94/NKG2C heterodimers present. Abnormal loss of CD161 expression is also frequent in NK-LGL. These patterns of CD94/NKG2A heterodimers and CD161 expression can serve as additional features to distinguish NK-LGL from reactive NK-cell expansions. CD94 expression has also been detected by reverse transcriptase–polymerase chain reaction in a subset of NK-cell lymphomas of the nasal type. In this neoplasm, CD94 expression seems to be an indicator of good prognosis.27

Conclusion

Characterizing NK-cell disorders can be problematic because these cells lack a single, unique, defining attribute. Furthermore, it is difficult to distinguish NK cells from cytotoxic T cells, particularly in paraffin-embedded material. Despite these challenges, a comprehensive approach to NK-cell immunophenotyping, including evaluation of NKRs, can enable the distinction of abnormal NK-cell expansions from cytotoxic T cells and reactive NK-cell populations.

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