Commentary

Immunoglobulin and T-Cell Receptor Gene Rearrangements: Minding Your B's and T's in Assessing Lineage and Clonality in Neoplastic Lymphoproliferative Disorders

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Antigen receptors (ARs), namely immunoglobulins on B cells and T cell receptors on T cells, are one of the central components of the adaptive immune response, providing the ability to recognize antigens in a humoral and cellular context, respectively. One of the major mechanisms underlying the generation of the remarkably diverse immune response, despite the rather limited number of genes encoding ARs, is the process of gene rearrangement. Other mechanisms contributing to this diversity include the deletion and insertion of N-nucleotides by the enzyme terminal deoxynucleotidyl transferase, which occurs early in lymphoid ontogeny in both B and T cells, essentially in tandem with the rearrangement process, and somatic hypermutation, which is restricted to the later, germinal center phase of B cell development. During the rearrangement of AR (immunoglobulin [IG] and T cell receptor [TCR]) genes, there is an apparent random shuffling and rejoining of the various segments of these genes. These segments are the variable (V), diversity (D), and joining (J) regions; V and J segments are present in all AR genes, with D segments present only in some. While the determination of V, D, or J segments to be used in a specific rearrangement is apparently stochastic, the mechanisms through which the actual rearrangement occur is not, in that specific heptamer and nonamer recombination signal sequences (RSS) flank these segments and that the process is initiated by a specific complex that includes RAG1 and RAG2 (collectively RAG, proteins of the recombination activating genes).¹

The prevailing dogma had been that only B cells rearrange their IG genes, and only T cells rearrange their TCR genes, and these rearrangements occur very early in lymphoid development. Consequently, the analysis of AR gene rearrangements ought to provide an extremely useful diagnostic laboratory tool for lineage determination. Indeed, there are numerous scenarios in which previ-

ously enigmatic—with regards to cell of origin—hematological malignancies, such as Hodgkin lymphoma, have had their heretofore-elusive lineages assigned based on such analyses.^{2,3} However, even more powerful than their putative role in lineage assignment, the analysis of AR genes has been central to the assessment of the clonality of lymphoproliferative disorders. Indeed, both in (genetic) isolation as well as in the context of more robust immunophenotypic tools, the limitations of AR genes as determinants of lineage have been exposed. The basis of their major utility in the documentation of monoclonality is that whereas a polyclonal population of lymphocytes will contain a heterogeneous admixture of cells, each with a different V-(N)-(D)-(N)-J rearrangement, a monoclonal population, having been derived from a single transformed cell, will spawn a homogeneous population of cells that evince an identical gene rearrangement. Typically, such populations can be distinguished easily from one another using standard molecular diagnostic approaches. Historically (ie, 10 years ago!), this could be done with Southern blots and currently using polymerase chain reaction (PCR). PCR analysis of the IG (in particular the IG heavy chain [IGH]) and TCR (in particular the TCR γ [TRG]) genes account for a large proportion of tests performed in clinical molecular diagnostic laboratories and provides valuable diagnostic information in the evaluation of atypical (neoplastic versus reactive) lymphoproliferations, albeit with well-known caveats regarding diagnostic specificity and sensitivity.⁴ In addition, the documentation of a monoclonal AR gene rearrangement

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provides a molecular fingerprint of the neoplasm, which can then be used for subsequent, and prognostically relevant, minimal residual disease assessment.^{5,6}

It is within this context of the frequent utility of these assays that the article by Tan and colleagues in this issue of *The Journal of Molecular Diagnostics*⁷ is of interest and warrants commentary. One of the take-home messages of their study is that, among T-cell lymphomas, it is not only in the biologically fascinating entity of angioimmunoblastic T-cell lymphoma that one is likely to encounter the apparent contradictory presence of a monoclonal *IGH* gene rearrangement in about one-third of such cases, but also in a similar proportion of cases of T-cell lymphomas in the "wastebasket" category of peripheral T-cell lymphoma, unspecified. Among others, this analysis highlights the perhaps growing and under-appreciated scenarios in which the presence of "cross-lineage" AR receptor gene rearrangements may be encountered and provides potentially confusing diagnostic laboratory results that may be difficult to interpret for both the uninitiated and experienced laboratorian. The term "cross-lineage" is not entirely satisfying, but as used in this discussion pertains to the presence of unexpected rearrangements, defined here by being contrary to what might be anticipated from the immunophenotype/lineage of the neoplastic cells under study. Broadly considered, there are two contexts in which such cross-lineage rearrangements may be encountered: those that occur in the *same* neoplastic cells and those that occur in *separate*, distinct populations of cells.

With regard to the former context, it has long been recognized that a subset of lymphoid neoplasms, clearly restricted to either B or T cell lineage based on unequivocal immunophenotypic data, harbor cross-lineage rearrangements, a phenomenon usually termed "lineage infidelity" (and sometimes "lineage promiscuity"). Interestingly, this is much more prevalent in immature or precursor neoplasms, as compared with more differentiated or peripheral neoplasms.⁸ For example, whereas ~70% of (immature) precursor B-cell lymphoblastic leukemias/lymphomas may contain monoclonal *TRG* rearrangements, only ~5 to 10% of cases of (mature) B-cell chronic lymphocytic leukemias harbor such cross-lineage rearrangements. The explanation for this wide difference is unclear, but one tenable hypothesis has been that of the putative timing of when, in the hierarchy of differentiation and maturation, a normal cell is transformed into a neoplastic cell. What this thesis proposes is that immature, precursor leukemias/lymphomas become neoplastically transformed at an early stage in their development, when the factors mediating AR rearrangements, including RAG, are (physiologically) active; this process then, perhaps, goes somewhat (pathologically) awry, with consequent cross-lineage rearrangement. This misdirected rearrangement may well underlie the actual transforming event, in that a number of chromosomal translocations associated with specific lymphomas and leukemias may be mediated by inappropriate RAG activity.⁹ The finding of RSSs flanking some of the genes involved in these translocations (other than the AR genes, which are typically partners in such events), as well as the presence of

N-nucleotides, inserted by deoxynucleotidyl transferase at the sites of genomic recombination, renders this view even more plausible. The reduced frequency of cross-lineage rearrangements in more mature, peripheral lymphoproliferative disorders, as compared with the immature blastic proliferations, may be due to the latter being transformed at somewhat later stages of lymphoid differentiation, long after the V(D)J segments have undergone physiological rearrangement, lessening the likelihood of a cross-lineage rearrangement.

While the reasons for these observations may be poorly understood, they provide a most convenient phenomenon for the study of minimal residual disease, particularly in the context of precursor lymphoblastic leukemias such as precursor B-cell acute lymphoblastic leukemia. The assessment of minimal residual disease here relies on the ability to detect at very low levels, using allele-specific reagents (primers and/or probes) and quantitative PCR, the clonogenic *IGH* gene rearrangement that defined the original precursor B-cell acute lymphoblastic leukemia, providing the ability to track sensitively (and specifically) the neoplastic clone following remission-induction therapy. However, as a testament to the wiliness of these malignant cells, these *IGH* rearrangements are often capricious, with the specific rearrangement that dominates (because such leukemias may actually be oligoclonal¹⁰) at diagnosis sometimes having disappeared at relapse, negating its utility in minimal residual disease assessment. For this reason, it has been recommended that to undertake minimal residual disease analysis optimally in the scenario of precursor B-cell acute lymphoblastic leukemia, two or more different AR loci, including *IGH* and *TRG*, should be tracked to increase the likelihood that one is tracking at least one stable, retained rearrangement that is present throughout the course of the disease.¹¹

In contrast to the aforementioned examples in which both *IG* and *TCR* monoclonal rearrangements coexist in the *same* cell, dual rearrangements may also occur in *different* cells residing in the same anatomical site and occasionally in the same pathological lesion. As Tan and colleagues confirm, the latter is prototypically evident in angioimmunoblastic T-cell lymphoma, but they also show that this is quite common in peripheral T-cell lymphoma, unspecified.⁷ In these two common T-cell lymphomas, a separate B cell clone presumably emerges in the milieu of immune dysregulation, which is well described in angioimmunoblastic T-cell lymphoma and in which the inferior prognosis is often associated with the development of opportunistic infections in this immunodeficient context. Here, the perturbed immunological background appears to be secondary to the T-cell lymphoma itself, rather than preempting the development of the lymphoma. This is in contrast to the usual context in which immunodeficiency-associated lymphomas develop, including acquired (HIV, posttransplantation) and congenital (primary/hereditary) immunodeficiency disorders. Nevertheless, EBV appears to play a common and prominent role in the B cell expansions in both the putative secondary immunodeficiency associated with these T-cell lymphomas as well as the noted primary immunode-

iciency situations. The histological and immunophenotypic features alone, of EBV+ B cells within a background of T-cell lymphoma, are typically considered sufficient to support the notion that they reflect distinct clones, harboring independent monoclonal *IGH* and *TCR* gene rearrangements, respectively. On occasion, this has been demonstrated using an assessment of laser capture microdissected tissues.¹² Future studies may be directed at evaluating additional T-cell lymphomas to determine whether this phenomenon is more widespread or is restricted to these more common subtypes of T-cell lymphoma.

In general, the B cell clones in these T-cell lymphomas are an interesting immunological passenger, and do not behave as independent malignancies. However, on occasion, overt B-cell lymphomas may emerge,¹³ in which case the term "composite lymphoma" may be applied. In addition to this apparently unusual occurrence in these T-cell lymphomas, with the B-cell lymphoma typically, but not always, developing after some period of time, two distinct lymphomas may appear in the same anatomical site *ab initio*. Thus, the literature is replete with numerous single case reports or small series of cases of, but a conspicuous paucity of current reviews on, *bona fide* composite lymphomas that arise simultaneously.^{14,15} In most reports, these reflect a combination of two B-cell lymphomas, either two different "non-Hodgkin" lymphomas, or a "non-Hodgkin" and a Hodgkin lymphoma. The coexistence of a B- and a T-cell lymphoma is less common; as might be expected, such cases typically harbor both a monoclonal *IGH* and a monoclonal *TCR* gene rearrangement, an occurrence that is the focus of this commentary. Interestingly, some so-called composite B-cell lymphomas are actually clonally related, with the two forms reflecting different manifestations of the same neoplastic clone. This may occur in one of two ways: when a single clonal cell diverges to generate two related but distinct clones or when a single neoplastic cell acquires a secondary mutation driving metamorphosis into another subtype of lymphoma, such as when an indolent lymphoma transforms into a more aggressive lymphoma. The latter pathway is what is believed to occur in large cell (Richter) transformation of chronic lymphocytic leukemia; however, comparative molecular genetic studies in one series actually indicate that in a subset of such cases, the two clones are unrelated, with the two neoplasms indeed reflective of a true composite lymphoma, rather than clonal evolution of one into another.¹⁶

In addition to the descriptions of distinct expansions of B cells in T-cell lymphoma, sometimes manifesting as unequivocal second malignancies (composite lymphomas), and in others not necessarily overtly malignant [Epstein Barr Virus (EBV)+ proliferations in angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphoma], there is a third scenario in which coexisting monoclonal *IG* and *TCR* gene rearrangements may be encountered. In these situations, the monoclonal *IGH* gene rearrangement is reflective of the *bona fide* B cell neoplasm, with the T cell clonality reflective of a restricted immune response to the former. Curiously, this is observed most commonly, and best described, in the con-

text of plasma cell neoplasms, suggesting some antigenic peculiarity of the neoplastic plasma cells.¹⁷ Here, the clonal T cells, which are typically CD57+ cytotoxic T cells, are demonstrated by using T-cell receptor β Southern blot analysis, spectratyping, or monoclonal antibodies.

Thus, there are a variety of distinct situations in which monoclonal *IG* and *TCR* gene rearrangements may coexist, either in the same neoplastic cell or in different cells, one or both of which may be overtly neoplastic. Knowledge thereof reinforces the notion that when a diagnostic molecular professional is confronted by such data, it is better to interpret these as markers of clonality, rather than of lineage, indicating that the dogma alluded to previously be treated with circumspection and that immunophenotypic features are perhaps more robust (but certainly not infallible) determinants of the latter. Indeed, five of the cases of T-cell lymphoma in the accompanying series harbored monoclonal *IGH* gene rearrangements, but lacked (detectable) *TRG* gene rearrangements⁷; in molecular diagnostic isolation, these findings might have been misinterpreted to be more consistent with a B cell neoplasm. Furthermore, the idea that "lineage infidelity" is a pathological phenomenon occurring only in neoplastic lymphocytes is flawed since it is well recognized, at least in the immunology literature, that this also occurs in normal lymphoid development.¹⁸ Finally, to ensure that both dogmas noted earlier are equally dispensed with, it is also known that the rearrangement process itself is not restricted to early lymphoid development, since receptor editing (of the *IGH* genes) also occurs late in B cell ontogeny, as an important mechanism in preventing the development of autoimmunity.¹⁹

Those who evaluate results of AR gene rearrangement studies are required, at first pass, to distinguish *polyclonality* from *monoclonality*, with these profiles typically, but certainly not always, consistent with reactive and neoplastic lymphoproliferations, respectively. Additional nomenclature has been adopted in the interpretation of PCR-based assays of AR gene rearrangements, especially when analyzed by capillary electrophoresis. Such terminology includes *oligoclonality* (consistent with a somewhat restricted immune response, typically but not universally reflected by the presence of three or more distinct peaks, reproducibly detected on repeat analysis, and sometimes in a polyclonal background) and *pseudoclonality* (because of the presence of a paucity of lymphocytes, typically in the absence of a polyclonal background but with the failure to reproduce the same peak on repeat analysis). This is not always straight forward, and it is likely that not all agree on the above definition of oligoclonality and, even more likely, that not all individuals interpret the same capillary electrophoresis tracings in the same way. What terminology might be used for the scenarios alluded to in this commentary? Perhaps *paraclonality* when the two clones are distinct and *biclonality* (or *bigenotypic*) when they occur in the same cell? Such neologisms are likely to be confusing and are to be avoided. However, proposed new terms and "pathobabble" aside, it is important that those as-

signed the responsibility of interpreting AR gene rearrangement analyses at least be aware of such potentially confounding results. And, finally, we should always consider the somewhat clichéd but nonetheless pithy mantra that what we do, at least in the world of molecular hematopathology, needs to be interpreted in the context of other pathological (including morphological, histological, cytogenetic, and immunophenotypic) and clinical findings.

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