Immunoglobulin and T-cell receptor gene rearrangement

Implications for the diagnosis of lymphoproliferative disorders

ANDREW J. FISHLEDER, MD

■ Recent studies have significantly broadened understanding of immunoglobulin production and T-cell receptor formation at the gene level. A limited number of genes can be rearranged during the course of B-cell or T-cell development to yield unique DNA sequences that will code for specific antibodies and T-cell receptor proteins. Southern blot hybridization analysis allows sensitive examination of lymphocyte DNA for the presence or absence of gene rearrangements. General mechanisms that underlie immunoglobulin and T-cell receptor rearrangement are reviewed, along with the diagnostic applications of detection of gene rearrangement by Southern blot hybridization technique.

□ INDEX TERMS: ANTIBODY FORMATION; ANTIBODY SPECIFICITY; LYMPHOPROLIFERATIVE DISORDERS; T LYMPHOCYTES □ CLEVE CLIN J MED 1989; 56:716–721

ADVANCES in molecular biology over the past decade have resulted in a wealth of information regarding the genes responsible for immunoglobulin production and T-cell receptor (TCR) formation. This discussion will first address the normal organization of the immunoglobulin gene system and the implications of the detection of immunoglobulin gene rearrangement in the diagnosis of lymphoproliferative disorders. It will then examine the TCR beta chain gene system and the role it plays in the specificity of T cells for foreign antigens.

The immunoglobulin molecule is well known to contain light chains and heavy chains, both of which contain variable and constant regions. The variable regions are responsible for the specificity of an individual antibody for a particular antigen while the constant regions code for sequences that allow recognition of heavy chain or light chain class, as well as functions common to antibodies of similar class. Scientists have been puzzled for many years about how the human genome, with its limited amount of DNA, can code for the tremendous antibody diversity required to provide an adequate immune system. It is now understood that the explanation is based upon the ability of a limited number of immunoglobulin genes to rearrange prior to the commitment of an individual B lymphocyte to immunoglobulin production.[1,2] The rearranged DNA sequence is responsible for the production of a unique antibody by a specific B-cell clone.

The kappa light-chain genes, lambda light-chain genes, and heavy-chain genes are found on chromosomes 2, 22, and 14, respectively.[3] In general, different types of genes are responsible for the production of segments of either the heavy-chain or light-chain portion

From the Department of Laboratory Hematology, The Cleveland Clinic Foundation. Submitted July 1987; accepted Feb 1988.

Address reprint requests to A.J.F., The Cleveland Clinic Foundation, 9500 Euclid Avenue, One Clinic Center, Cleveland, Ohio 44195.

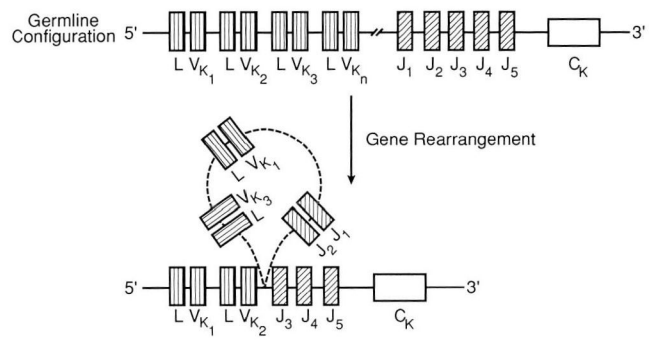

FIGURE 1. Diagram illustrating the rearrangement of variable region (V) and joining region (J) genes of the kappa light chain gene locus on chromosome 2. The dotted loop in lower portion of figure indicates segment of DNA deleted following gene rearrangement.

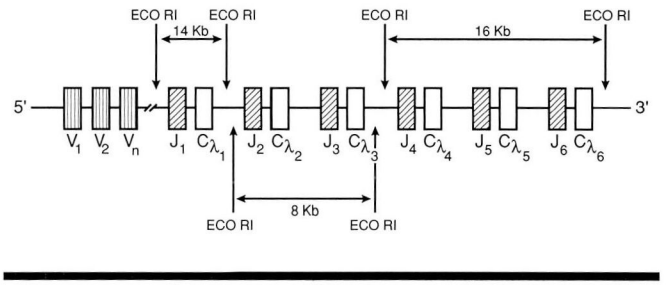

FIGURE 2. Diagram of *Eco*RI restriction sites at lambda light-chain gene locus. From Arnold et al.[11]

of an immunoglobulin molecule. These are referred to as the "variable region" (V) genes, "diversity region" (D) genes, "joining region" (J) genes, and "constant region" (C) genes.[3] Within each gene cluster (kappa, lambda, or heavy-chain), there are numerous V-genes, J-genes, and one or more C-genes. In addition, each V-gene is proceeded by a "leader" (L) gene, which is responsible for the production of a short protein segment that is thought to be necessary for the transport of an antibody molecule through the B-cell membrane.

Prior to the commitment of an immature lymphoid cell to either B-cell or T-cell lineage, the immunoglobulin genes remain in an unrearranged or "germ line" configuration. At some point early in the maturation of a B lymphocyte, there is rearrangement of the immunoglobulin-gene DNA to yield new gene sequences.[1] In this way, antibody diversity is achieved. More specifically, in the kappa light-chain gene system, there are numerous (>100) V-genes, five J-genes, and one C-gene (*Figure 1*). Upon rearrangement, a single V-gene positions itself adjacent to one of the five J-genes and the intervening DNA is deleted. The C-gene, which is located farther along the chromosome, remains in its germ-line configuration. Following V-J rearrangement the new DNA sequence and constant region sequence are transcribed into a precursor mRNA molecule, which is further edited and spliced into a mature mRNA segment. This final mRNA product can then be translated to yield the light-chain protein. The numerous possible combinations of V-genes and J-genes are further expanded by the ability of an individual V-gene to be transposed adjacent to an individual J-gene at one of multiple sites.[1] This potential for a multitude of V-J recombinations and therefore unique V-J sequences accounts for the tremendous diversity of light-chain amino acid sequences.

The lambda light-chain gene locus is similar to the kappa gene locus except that there appear to be fewer V-genes and there are six C-genes, each of which is linked to an individual J-gene (*Figure 2*).[4] Although the lambda gene locus is organized somewhat differently from the kappa gene locus, gene rearrangement is still responsible for gene product diversity.

The heavy-chain gene locus also possesses multiple V-genes, as well as six J-genes, and separate C-genes that code for each heavy-chain subclass (mu, gamma$_1$, gamma$_2$, etc) (*Figure 3*).[4] Additionally, a small group of D-genes is located between the V-gene and J-gene clusters. During heavy-chain gene rearrangement an individual D-gene is first rearranged adjacent to a J-gene, bypassing the intervening DNA prior to transposition of a V-gene adjacent to the D-J segment.

Following the initial rearrangement of the V, D, and J-genes, the mRNA that is transcribed from the heavy-chain gene locus includes transcripts of the constant-region genes that code for mu and delta specificity. This is in keeping with the knowledge that a B lymphocyte initially produces IgM and IgD immunoglobulin molecules. During the course of an immune response, this is typically followed by a switch to IgG or IgA production, although the antibody specificity remains intact. Heavy-chain class switching is now recognized to be a result of rearrangement of the C-genes, triggered by an as yet unidentified mechanism, with retention of the previously rearranged V-D-J sequence (*Figure 3*) accounting for retained antigen recognition.

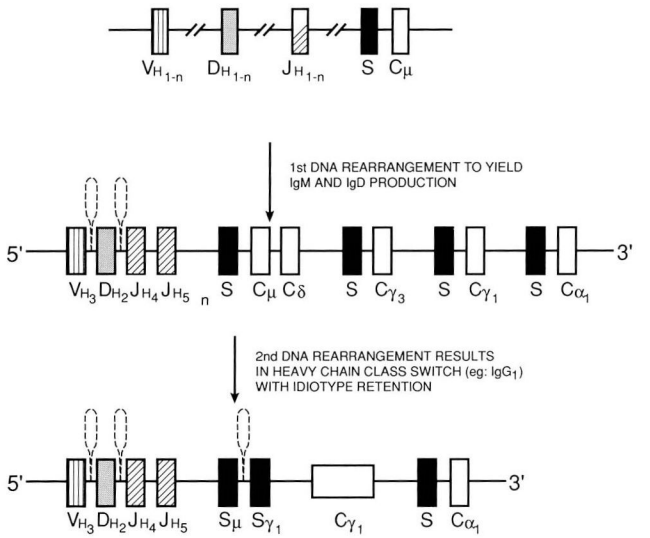

FIGURE 3. Diagram illustrating the rearrangement of variable region (*V*), diversity region (*D*), joining region (*J*), and constant region (*C*) genes of the heavy-chain gene locus on chromosome 14. Center portion of figure indicates initial rearrangements of variable and diversity region genes as part of primary immune response. Lower portion of figure indicates rearrangement of heavy-chain genes resulting in heavy-chain class switching. From Waldmann et al.[18]

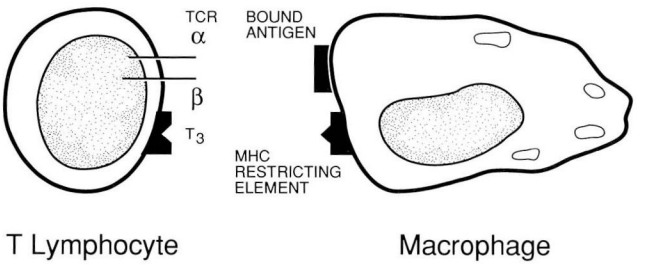

FIGURE 4. T lymphocyte with dimeric T-cell receptor (*TCR*) and T_3 receptor on surface. T_3 receptor recognizes major histocompatibility complex (*MHC*) restricting element on macrophage. TCR recognizes bound antigen on macrophage.

Finally, it is now understood that the individual immunoglobulin gene loci undergo an ordered sequence of rearrangement: the heavy-chain genes rearrange prior to the light-chain genes and the kappa light-chain genes rearrange prior to the lambda light-chain genes.[5] Furthermore, if the kappa light-chain gene rearrangement yields a functional DNA sequence then the lambda light-chain genes do not undergo rearrangement.[6] However, if the kappa light-chain gene rearrangement results in a nonfunctional rearrangement or deletion of the genes then the lambda light-chain genes will rearrange. This presumably explains the 2–3:1 kappa to lambda light-chain ratio that is normally encountered in polyclonal B-cell proliferations, as well as the excess of lymphomas producing kappa light chains relative to those producing lambda light chains. (It should also be recognized that when an individual gene locus, such as the heavy-chain genes or kappa light-chain genes, undergoes rearrangement, only one of the particular chromosome pair attempts rearrangement initially. Should that rearrangement be successful then the genes located on the second chromosome of the pair will remain in the germ-line configuration. Only if the rearrangement of the genes on the first chromosome is unsuccessful will the second chromosome attempt rearrangement.)

Unlike B cells, which produce immunoglobulins that recognize free antigens, T cells recognize antigens that are bound to the surface of antigen-presenting cells such as macrophages and monocytes. The ability of a T cell to recognize such foreign antigens is dependent upon a complex of two receptors on the T-cell surface (*Figure 4*).[7] The T3 antigen recognizes major histocompatibility complex (MHC) restricting elements on the presenting cell surface and this allows the TCR to recognize the foreign antigen. It is the TCR, therefore, that must have the ability to recognize specifically a variety of antigens in a manner similar to the immunoglobulin molecule.

The TCR is a dimer composed of an alpha chain and a beta chain. Each chain has both constant and variable regions similar to the component chains of the immunoglobulin molecule; the combination of the variable regions of the alpha and beta chains is responsible for antigen recognition. Although the genes coding for both the alpha and beta chains have been characterized, evaluation of the beta-chain gene family is more widely used to recognize T-cell clonal expansion because it rearranges prior to the alpha-chain gene family. For this reason, the remainder of this section will deal with the TCR beta-chain genes.

The TCR beta-chain genes are located on chromosome 7q and are organized somewhat differently from the immunoglobulin loci (*Figure 5*).[7] The V-genes, of which there are approximately 50, are located in the 5′ direction from all of the D, J, and C-genes. There are

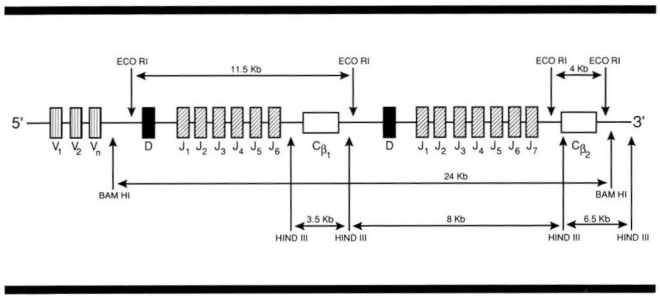

FIGURE 5. Diagram illustrating *Eco*RI, *Bam*HI, and *Hin*dIII restriction sites of the T-cell receptor beta-chain gene locus.

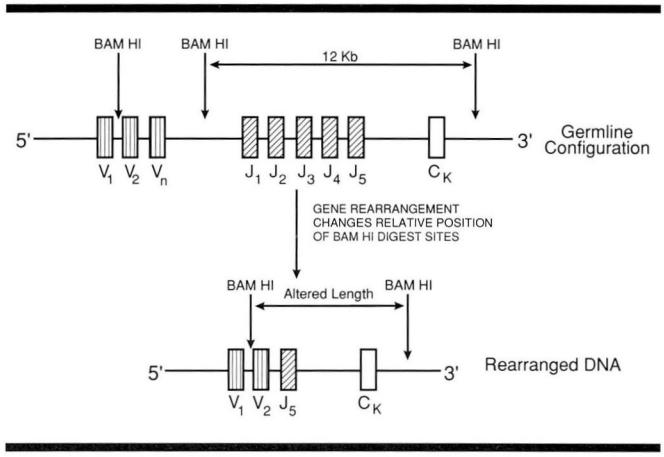

FIGURE 6. Diagram illustrating alteration of *Bam*HI-restricted fragment size at kappa light-chain gene locus following gene rearrangement. This figure is a modification of Figure 1 from Arnold et al.[11]

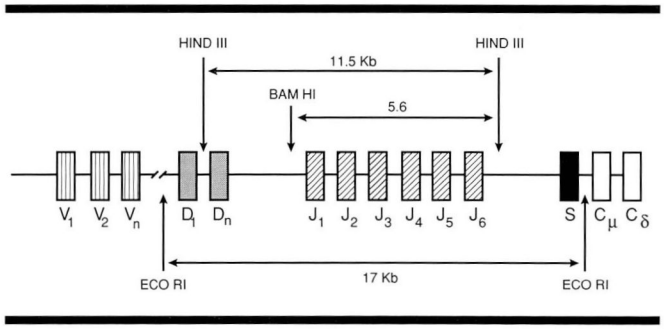

FIGURE 7. Diagram of *Eco*RI, *Bam*HI, and *Hin*dIII restriction sites of heavy-chain gene locus.

two C-genes, $C\beta_1$ and $C\beta_2$, which are located approximately 10 kb apart. There are six and seven J-genes, respectively, in the 5′ direction from each C-gene and adjacent to the 5′ end of each of these J segments is a single D-gene. In contrast to the immunoglobulin genes, in which there is a sequential ordering of V, D, J, and C-genes, in the TCR beta chain gene system there is a single group of V-genes upstream from clusters of D, J, and C-genes. Regardless of this minor dissimilarity, rearrangement of the V, D, and J-genes is again responsible for the creation of new DNA sequences that code for unique beta-chain proteins. Also in a manner analogous to the immunoglobulin genes, the TCR genes undergo sequential rearrangement; the T-cell beta-chain genes are rearranged and expressed prior to expression of the alpha-chain genes.

Detection of immunoglobulin and TCR gene rearrangements is accomplished by restriction endonuclease digestion of DNA followed by Southern blotting and hybridization to probes specific for individual genes. Those most commonly used include probes for the C-genes of the kappa, lambda, or TCR beta-chain gene loci or the joining region gene of the heavy-chain locus. In the germ-line configuration, specific restriction endonucleases yield identifiable gene segments of constant lengths. For example, *Bam*HI digestion of the kappa gene locus yields a 12-kb DNA segment when hybridized with the kappa C-gene probe (*Figure 6*). In contrast, the same *Bam*HI digestion would yield a 24-kb DNA segment when hybridized to the TCR beta-chain C-gene probe (*Figure 5*). The heavy-chain genes are more commonly examined following digestion with *Eco*RI or a combination of *Bam*HI and *Hin*dIII to yield 17-kb and 5.6-kb germ-line segments, respectively, when hybridized to the probe for the J-genes (*Figure 7*). (Caution must be exercised when using *Hin*dIII digestion alone [11-kb germ-line], however, because of polymorphisms observed in the 5′ direction from the J_H region.)[8] Finally, when examining the lambda light-chain gene locus with a probe for the C-genes an *Eco*RI digestion is most typically used and, in contrast to the previously mentioned simple germ-line configurations, yields three DNA segments, most typically of 8 kb, 14 kb, and 16 kb (*Figure 2*). However, this is further complicated by polymorphisms within the population that can yield additional germ-line fragment sizes of 18 kb, 21 kb, and 23 kb in the lambda gene system.[9]

In the TCR beta chain gene system, *Eco*RI and *Hin*dIII restriction endonucleases are commonly used to detect rearrangements involving $C\beta_1$ and $C\beta_2$, respectively (*Figure 5*). Germ-line bands following *Eco*RI digestion are 11.0 and 4.0 kb, however, an additional 9.5-kb band may be identified due to an *Eco*RI site that is resistant to

endonuclease digestion.[4] Recognition of this problem is important to prevent misinterpretation of this "new fragment" as a true gene rearrangement.

When the V, D, and J-genes rearrange during the course of B-cell or T-cell maturation and an altered DNA sequence is formed, sites of restriction endonuclease cleavage are shifted, resulting in DNA digestion products that contain DNA fragments of altered length (*Figure 5*). This unique gene sequence is then a marker of that specific B-cell or T-cell clone.[4]

This information is very useful in the diagnosis of lymphoproliferative disorders since the technique of restriction endonuclease digestion, Southern blotting, and DNA hybridization is sensitive enough to detect a clonal expansion of only 1%–3% of the DNA in a sample. In typical reactive processes reported to date, a multitude of B-cell or T-cell clones proliferate, with no individual clone more than 1% of the total lymphocyte population. Only residual germ-line DNA is identifiable and no rearrangements can be detected.

On the other hand, B-cell or T-cell clonal expansions associated with lymphoproliferative disorders commonly account for more than 1%–3% of a lymphoid population in a lymph node or in the peripheral blood. When examining the DNA from a lymphoma, then, one would expect to identify on a Southern blot a DNA segment of altered size (non-germ-line) indicating a monoclonal proliferation.[10,11] This is particularly useful in the diagnosis of lymphoproliferative disorders in cases that histologically appear to represent partial involvement of a lymph node by lymphoma, in SIg-negative B-cell lymphomas, or in cases where immunophenotyping yields equivocal results. In addition, detectable immunoglobulin or TCR rearrangement can help to differentiate lymphomas from other poorly differentiated nonlymphomatous neoplasms.

Unlike the B-lymphocyte system, in which uniform surface immunoglobulin can indicate monoclonality, there is no antigenic marker of clonality in the T-cell system. However, TCR beta-chain gene rearrangement does indicate a monoclonal T-cell proliferation[12,13] and therefore is helpful in identifying T-cell neoplasms. In particular, examination of the TCR beta-chain genes can be useful in the diagnosis of mycosis fungoides and in the subsequent detection of Sezary cells in enlarged lymph nodes in those patients.[14] Although the histologic distinction between dermatopathic lymphadenopathy and Sezary-cell involvement of a lymph node can be quite difficult, the detection of a rearranged TCR beta-chain gene segment would suggest Sezary involvement of the lymph node, especially if that new DNA fragment were of the same kilobase length as that detected in the primary mycosis involvement of the skin.

Although we have equated monoclonality with neoplastic proliferations in the past, more sensitive detection of gene rearrangements by Southern blotting techniques may identify some conditions that are not clinically malignant but that are associated with clonal lymphocyte proliferations. We cannot assume that monoclonality at the gene level has the same clinical implication as immunologic monoclonality. This can be illustrated by immunoglobulin gene rearrangements that have been identified in the benign lymphoepithelial lesion of the salivary gland typically found in association with Sjögrens syndrome. This has previously been considered to be a benign polyclonal lymphocyte proliferation but is now recognized as possessing a monoclonal B-cell component.[15] Similarly, the disorder lymphomatoid papulosis has been considered a chronic inflammatory process involving the skin, but has now been demonstrated to possess TCR beta-chain gene rearrangements indicating a monoclonal T-cell component.[16] Finally, we now recognize that immunocompromised individuals, such as transplant patients, may develop monoclonal or oligoclonal lymphocytic proliferations that resolve when immunosuppressive agents like cyclosporine are removed.[17]

CONCLUSION

In conclusion, molecular biologic advances have significantly broadened our understanding of the immunoglobulin and TCR gene systems and have provided the tools necessary to allow identification of monoclonal B-cell and T-cell proliferations at the gene level. The sensitivity of these techniques can provide a useful adjunct to the diagnosis of lymphoproliferative disorders; however, we must be cautious and recognize that the detection of gene rearrangement does not necessarily equate with malignant lymphocytic clonal expansion.

REFERENCES

1. Leder P. The genetics of antibody diversity. Scientific Am 1982; **246:(5):**102–115.
2. Korsmeyer SJ, Hieter PA, Sharrow SO, Goldman CK, Leder P, Waldmann TA. Normal human B cells display ordered light chain gene rearrangements and deletions. J Exper Med 1982; **156:**975–985.
3. Goldman JN, Goldman MB. The genetics of antibody production. JAMA 1984; **251:**774–786.
4. Waldmann TA. The arrangement of immunoglobulin and T cell

receptor genes in human lymphoproliferative disorders. [In] Dixon FJ, et al, eds. Advances in Immunology. Vol. 40. San Diego, Academic Press, 1987, pp 247–321.
5. Korsmeyer SJ, Hieter PA, Ravetch JV, Poplack DG, Waldmann TA, Leder P. Developmental hierarchy of immunoglobulin gene rearrangements in human leukemic pre-B cells. Proc Natl Acad Sci 1981; **78:**7096–7100.
6. Hieter PA, Korsmeyer SJ, Waldmann TA, Leder P. Human immunoglobulin kappa light-chain genes are deleted or rearranged in lambda-producing B cells. Nature 1981; **290:**368–372.
7. Minden MD, Mak TW. The structure of the T cell antigen receptor genes in normal and malignant T cells. Blood 1986; **68:**327–336.
8. Fey MF, Wainscoat JS. DNA polymorphism 5′ to the JH region of the human immunoglobulin heavy chain gene and immunoglobulin gene rearrangements in leukemia. Am J Clin Pathol 1988; 187–189.
9. Hieter PA, Hollis GF, Korsmeyer SJ, Waldmann TA., Leder P. Clustered arrangement of immunoglobulin λconstant region genes in man. Nature 1981; **294:**536–540.
10. Cleary ML, Chao J, Warnke R, Sklar J. Immunoglobulin gene rearrangement as a diagnostic criterion of B-cell lymphoma. Proc Natl Acad Sci 1984; **81:**593–597.
11. Arnold A, Cossman J, Bakhshi A, Jaffe ES, Waldmann TA, Korsmeyer SJ. Immunoglobulin-gene rearrangements as unique clonal markers in human lymphoid neoplasms. N Engl J Med 1983; **309:**1593–1599.
12. Flug F, Pier-Giuseppe P, Bonetti F, Knowles DM II, Dalla-Favera R. T-cell receptor gene rearrangements as markers of lineage and clonality in T-cell neoplasms. Proc Natl Acad Sci 1985; **82:**3460–3464.
13. Knowles DM II, Pier-Giuseppe P, Dalla-Favera R. T-cell receptor beta chain gene rearrangements: genetic markers of T-cell lineage and clonality. Hum Pathol 1986; **17:**546–551.
14. Weiss LM, Hu E, Wood GS, et al. Clonal rearrangements of T-cell receptor genes in mycosis fungoides and dermatopathic lymphadenopathy. N Engl J Med 1985; **313:**539–544.
15. Fishleder A, Tubbs R, Hesse B, Levine H. Uniform detection of immunoglobulin-gene rearrangement in benign lymphoepithelial lesions. N Engl J Med 1987; **316:**1118–1121.
16. Weiss LM, Wood GS, Trela M, Warnke RA, Sklar J. Clonal T-cell populations in lymphomatoid papulosis: evidence of lymphoproliferative origin for a clinically benign disease. N Engl J Med 1986; **315:**475–479.
17. Cleary ML, Warnke R, Sklar J. Monoclonality of lymphoproliferative lesions in cardiac-transplant recipients. N Engl J Med 1984; **310:**477–482.
18. Waldmann TA, et al. Molecular genetic analysis of human lymphoid neoplasms. Ann Intern Med 1985; **102:**497–510.