A Measure of Genomic Instability and Its Relevance to Lymphomagenesis

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Abstract

A recent pilot study that we performed on 12 individuals who are involved in the cultivation and processing of grains and legumes suggests to us that we may have in hand a relatively quick, inexpensive, and highly sensitive assay that identifies individuals at increased risk for the development of lymphoid malignancy. The generation of this assay evolved from our interest in the causes and consequences of lymphocyte-specific chromosomal aberrations.

Every human individual carries, within his/her peripheral blood T-cell population, cells that differ from the normal 46XY or 46XX chromosomal complement. In general these chromosomal aberrations are not random but are reproducibly seen in all individuals and are T-cell specific. They involve combinations and permutations of chromosomal breaks at two points on chromosome 7 (bands p13 and q35) and two points on chromosome 14 (bands q11.2 and q32.3). With the advent of gene mapping it was quickly realized that these chromosomal breakpoints were within the same bands to which were localized the TCRγ (7p13), TCRβ (7q35), TCRα/β (14q11.2), and Ig heavy chain (14q32.3) loci (1). Shortly thereafter it was demonstrated that these aberrations are formed by site-specific recombination between V segments from one immune receptor locus and D or J segments from a different one, forming hybrid immune receptor genes (2–4). The existence of these hybrid receptors has implications for our understanding of (a) the repertoire of our immune response, (b) the chromatin accessibility of different immune receptor loci at different stages of lymphocyte development, and (c) the mechanism of action of the V(D)J recombinase enzyme complex. Some of the hybrid receptors are indeed in-frame, transcribed, and membrane associated (2–5).

Other investigators have demonstrated that experimentally created hybrid genes are capable of functioning in antigen-binding assays and mitogenic triggering (6–8). It remains to be elucidated what such hybrid receptors may mean in terms of positive and negative selection of lymphocytes with certain binding specificities. It is also unclear what the ramifications are of forming a dimer between two chains, parts of which are not usually considered as juxtaposable. For such hybrid receptors to form it is necessary for both loci involved to have been simultaneously accessible to the action of the recombinase complex and that the recombinase complex be capable of handling the increased number of DNA ends that such hybrid formation necessitates. These issues raise questions about the ordered sequential activation of immune receptors during B- and T-cell development, the topographical and topological organization of DNA in the interphase nucleus, and the number of recombinases available to act at a particular site of recombination.

The relevance of the formation of these hybrid receptors to lymphomagenesis initially emerged with our realization (4) that their frequency was increased about 100-fold over baseline in patients suffering from the autosomal recessive disease AT. This was assessed by a PCR-based assay that we developed in the laboratory. In this assay we specifically screen for one of the T-cell-specific chromosomal abnormalities, an inversion of chromosome 7, inv(7)(p13q35). We have shown that this inversion is caused by site-specific recombination between a TCRγ V segment and a TCRβ J segment. The mechanism of formation of hybrid genes is mediated by the V(D)J recombination complex. The cells that carry these morphological aberrations are polyclonal, and thus in AT patients the increased frequency of these aberrations actually represents 100-fold more independent events that generate them. AT is a disease of protein manifestations including progressive cerebellar ataxia, oculocutaneous telangiectasia, immunodeficiency, radiosensitivity, and a predisposition to the development of certain cancers, particularly lymphoid malignancies. The lymphoid malignancies from which these patients suffer represent the same spectrum as that of the general North American population, but in AT patients there is an approximately 100-fold increased risk. Thus the increased risk of lymphoma is paralleled by an increased frequency of hybrid gene formation. The same V(D)J recombinase-mediated mechanism has been implicated in the formation of the cancer-associated chromosomal aberrations which are seen in and believed to contribute to the majority of lymphomas from which these patients suffer.

Shortly after publication of the above study we became aware of another population with an increased risk of development of lymphoid malignancy. This population did not bear a genetic predisposition but rather appeared to have an increased risk on the basis of environmental or occupational exposure, namely agricultural workers and grain millers (9–13). Cytogenetic analysis of the phytohemaglutinin-stimulated (predominantly T-cell) peripheral blood from these individuals revealed a pattern of chromosomal aberration that was reminiscent to us of patients with AT. It seemed to show an exaggeration of the "innocent" juxtapositions of those regions on chromosomes 7 and 14 that had been associated with interlocus V(D)J recombination events. Having developed our PCR-based assay for studying the frequency and clonality of one such aberration, inv(7), we applied it to this population of agriculture workers and "control" individuals not involved in the growing and processing of agricultural products. We have demonstrated that this population of agriculture workers does indeed have an increased frequency of hybrid gene formation. We looked again at the occurrence of the inv(7). (There is nothing special about studying the inv(7). This particular aberration was chosen for this assay because of the ease of developing PCR primers for studying it. We could just as well have studied any T-cell-specific "innocent" chromosomal aberration, e.g., the t(7;14)- (p13; q11.2) or any of the other possible combinations of 7 and 14 mentioned above.) The frequency of formation of the inv(7) was significantly higher in the agriculture workers than in the

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3 The abbreviations used are: TCR, T-cell receptor; PCR, polymerase chain reaction; AT, ataxia-telangiectasia.
4 I. R. Kirsch and C. Rittershouse, unpublished observations.
normal population, although less than in the AT population. The incidence of hybrid gene formation was directly correlated with amount of exposure to mixed pesticides, herbicides, and fumigants. There was a seasonal variation to the occurrence of hybrid genes. The increased frequency for this population was seen only during the summer growing season. Individuals tested 3–4 months prior to or following "exposure" did not, as a population, have an increased frequency of hybrid genes. We have not yet been able to correlate hybrid gene formation with a particular agent to which these individuals are exposed.

Thus two populations with an increased risk of development of lymphoid malignancy have an increased frequency of formation of "innocent" T-cell-specific chromosomal aberrations, aberrations mediated by the action of the V(D)J recombinase complex, the same complex involved in the generation of the malevolent translocations most frequently associated with the types of lymphomas that these populations develop. One population carries a genetic predisposition to lymphoma. The other population has an acquired increased risk of lymphoma development. The bulk of lymphomas that occur in these populations are of B-cell lineage. For the ease of our assay we looked primarily at the T-cell population. This is not a drawback to the assay. The hybrid genes we are looking for are markers of an increased risk, not evidence of lymphoma itself. The same mechanism that causes an inv(7) in T-cells causes the "innocent" B-cell-specific t(2;14) translocation which involves a hybrid Igα/IgH gene (14, 15). What we believe we are likely to be observing is a change in chromatin accessibility (for a discussion of locus accessibility and V(D)J recombination see Ref. 16) caused either genetically or environmentally which makes the immune receptor loci more "accessible" to the action of the recombinase complex and thus increases genomic instability in lymphocytes. This increased "accessibility" is not restricted to immune receptor loci but occurs across the genome, making various growth-affecting genes more "accessible" as well. In AT patients this accessibility is a constant, a result of the fundamental defect in AT. In agriculture workers the AT defect is transiently mimicked during exposure to certain agents. It is at that time of exposure that one sees an increase in hybrid gene formation, and at that time of exposure that we would postulate that an individual would be at increased risk for the development of a malevolent chromosomal aberration that could sow the seed for subsequent clinical development of lymphoma.

The work described above represents a pilot study. Clearly, there is a need to test the reproducibility and efficacy of this assay in a prospective study. If the results continue to maintain the relationships described above then the potential uses of this assay would include (a) screening of individuals or populations for risk of lymphoma development. In those individuals who tested "positive" in this assay, a physician might want to monitor them more frequently or more sensitively for the development of lymphoid malignancy. (b) Screening of populations for exposure to agents with lymphomagenic potential. (c) If an in vitro correlate of this assay can be developed (perhaps by using a cell line with the potential to undergo V(D)J-mediated recombination) it might be possible to screen for certain lymphomagenic or mutagenic compounds in the laboratory. (d) If an agent or agents can be identified in the population of agriculture workers that accounts for their increased frequency (as a population) of hybrid gene formation, then the study of the mechanism of action of that agent may provide insight into the fundamental defect in AT and the issue of chromatin accessibility in general.

References

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