Trans-rearrangements and the risk of lymphoid malignancy*

I. R. Kirsch
National Cancer Institute – Navy Medical Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

Summary

Background: Antigen receptor 'trans-rearrangements' occur in all individuals and represent a particular type of genetic instability whose mechanism, V(D)J recombination, is the same as that required for the development of a normal immune response.

Design: We have measured the level of trans-rearrangements in a variety of populations characterized by increased risk for the development of lymphoid malignancy. The human populations studied include those with an inherited predisposition to lymphomagenesis (ataxia-telangiectasia patients), as well as populations at increased risk because of an occupational (agriculture workers) or iatrogenic (Hodgkin's disease patients) exposure. In addition, we have developed a mouse model for the more controlled analysis of these events.

Results: There is a correlation between the absolute number of trans-rearrangements (as a population mean or median) and risk of lymphoma, whether that risk is based on an inherited predisposition or acquired exposure.

Conclusion: This assay may serve as an easily measurable biomarker of lymphoma risk. If so, it is more than a fortuitous biomarker since the same mechanism responsible for the formation of trans-rearrangements is, at least in part, responsible for the majority of presumably 'malevolent' translocations associated with the transformation of lymphocytes.

Key words: biomarkers, genetic instability, lymphomagenesis, trans-rearrangements, V(D)J recombination

Introduction

Genetic instability is a fundamental fact of life. It is the causative force of evolution, a critical factor in the normal development of organisms from bacteriophage to humans, and the basis of malignant transformation. Every cancer that has been susceptible to detailed molecular analysis has been found to carry alterations in its DNA that distinguishes it from the nonmalignant tissue from which it arose. These point mutations, amplifications, deletions, or translocations are precisely the 'tumor-specific markers' that have been the object of investigative search for over a century.

Thus, the propensity to develop cancer can be viewed as based on the tendency to genetic instability. This tendency is the combination of one's inherited ability to, for example, replicate one's DNA with fidelity, detoxify carcinogens, recognize and repair DNA damage, etc., and one's exposure to destabilizing agents in the environment. If it were possible to measure this tendency it would be possible to assess individual or population risk, at a moment or over time, for the development of cancer.

The breakage and rejoining of DNA encoding variable (V), diversity (D), and joining (J) segments within immunoglobulin and T-cell receptor loci represent a particular kind of genetic instability. The DNA in a mature lymphocyte is no longer the same as that in a stem cell or cell of a different lineage. Within the lymphocyte, irreversible site-specific rearrangement events have taken place that have taken the disparate V, D, and J segments and brought them into contiguity [for review see 1]. These rearrangements can take place between as well as within the antigen receptor loci [2–7]. In humans, they are the cause of the characteristic recurring chromosomal inversions and translocations that have been repeatedly noted by cytogeneticists doing routine karyotyping of 'normal' peripheral blood lymphocytes [8]. These 'trans-rearrangements' occur at a level high enough to be easily observable by a PCR-based amplification assay (see below) but low enough to be amenable to comparison between individuals or populations. Thus, they are potential biomarkers of this particular type of genetic instability.

A quantitative assay of trans-rearrangements

The prototype trans-rearrangement that we have studied involves the site-specific juxtaposition of a variable segment from the TCRγ locus to a joining segment from the TCRβ locus. In humans, this causes a pericentric inversion of chromosome 7. The assay involves a nested PCR amplification using primers from the TCRγV coding region and from the intervening sequence flanking the 3' end of the TCRβJ segment closest to the TCRβ constant

* The US Government right to retain a non-exclusive, royalty-free licence in and to any copyright is acknowledged.
with a number of other genes that are believed to function in signal transduction or response to DNA damage [13]. The defective gene in AT patients, ATM, shares a PI3-kinase motif to both these probes to be considered a trans-rearrangement by this analysis. We have verified this assay by cloning and sequencing the amplified products (see below).

**Trans-rearrangements in selected human populations**

We have studied the level of TCRγV-TCRβJ trans-rearrangements in a number of human populations. Our studies to date suggest that at any given time 95% of the general population (based on a summation of data from our various 'control' groups) will have less than one such trans-rearrangement per 30000 peripheral blood monoclonal cells. Cytogenetic data on a much more limited number of total metaphases had suggested the frequency of inversion of chromosome 7 at approximately one in 10000 [9, 10]. Given that only a proportion of all inversions of chromosome 7 would involve the precise TCRγV from which the primers are taken, these two estimates appear to be in quite close agreement. In pilot studies, it has become clear, however, that certain populations have levels of trans-rearrangements that significantly exceed this frequency.

**Ataxia-telangiectasia**

Ataxia-telangiectasia (AT) is an autosomal recessive disease of protein manifestations including progressive cerebellar degeneration, oculocutaneous telangiectasia, premature aging, immunodeficiency, radiosensitivity, and a predisposition to develop cancer [11]. While individuals who suffer from this disease have a higher incidence of whatever malignancies are prevalent in the society in which they live, they are markedly predisposed to the development of lymphoid malignancies [12]. The defective gene in AT patients, ATM, shares a PI3-kinase motif with a number of other genes that are believed to function in signal transduction or response to DNA damage [13]. Individuals with AT have a 10–100-fold increase in the absolute number of trans-rearrangements compared to a non-AT population [6, 7]. This increase is polyclonal, and similar in magnitude to the increased risk of lymphoid malignancy in this group. Here, the presumed 'destabilizing' influence is inherent and constant.

**Agriculture workers**

There is a leukemia/lymphoma 'belt' in the midwestern United States that appears to center more in rural than urban sites [14]. Farmers in this region appear to have a 3–7-fold increased risk for the development of NHL. We studied a population of individuals involved in farming and grain milling from this area and discovered a markedly increased level of trans-rearrangements [15]. The level of trans-rearrangements was roughly correlated with the level of pesticide exposure and showed a seasonal variation, going up in the summer when the exposure was occurring and returning to baseline in the winter months.

**Hodgkin's disease patients undergoing chemotherapy**

Individuals who have been treated for Hodgkin's disease have an increased risk for the subsequent development of secondary malignancies of which hematopoietic malignancies predominate in the first five to ten years following therapy [16, 17]. We have studied the level of trans-rearrangements in patients receiving ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) or EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin) [18]. The absolute number of trans-rearrangements increased in this population during the course of therapy and returned to baseline within six months of cessation of treatment. It is not known whether the starting level, ending level, or 'area under the curve' (or none of the above) might be of prognostic significance with regard to the development of a subsequent malignancy in these individuals.

**Processing samples for molecular epidemiologic studies**

In the previous section, we described a series of pilot studies that suggest that there is a relationship between the absolute number of trans-rearrangements found in peripheral blood lymphocytes and individual or population risk of lymphoma. The verification of these studies will require large-scale molecular epidemiologic efforts in which quantitation of exposure to suspected 'destabilizing' agents can be more precisely determined and for which long-term outcome follow-up information will be gathered. Implementation of such field studies may be aided by our refinement of this assay so that it can be performed on DNA extracted from 'finger-stick' blood placed onto filter papers [19]. Such simplification of the blood sampling and sample storing logistics makes the use of this or related assays much more economically feasible.
A murine model of trans-rearrangements

While awaiting the results of long-term studies in human populations, we have developed a murine model of trans-rearrangements in order to be able to study this phenomenon in a setting in which we could define and control the genetic and environmental contributions to this type of instability. We have duplicated the dose and timing of ionizing radiation that had been previously shown to be leukemogenic in the C57Bl/6 inbred strain of mice [20, 21] and found an increase in the level of trans-rearrangements (M. Hale, F. Lista, I. Kirsch, unpublished results). The most remarkable effect, however, came from experiments in which newborn SCID mice were subjected to a low dose (100 cGy) of ionizing radiation. This exposure had been previously shown to result in thymic proliferation and a wave of TCRβ intralocus rearrangement in these otherwise immunodeficient and V(D)J recombination-defective animals [22]. The stimulus was extremely carcinogenic, every mouse so irradiated succumbing to thymic lymphoma by 20 weeks of age. When trans-rearrangements in the thymus were assayed within one to two weeks following the irradiation (F. Lista, V. Bertness, C. Guidos, J. Danska, I. Kirsch, submitted for publication), there was a remarkable increase in the absolute number of these events. As many as one of every 100–200 thymocytes carried such a trans-rearrangement. As in the human studies, these trans-rearrangements were polyclonal. About 50% of them were in-frame at the genomic level and therefore capable of being translated into a functional ‘hybrid’ peptide. This percentage is not that different from the percentage of intralocus TCRβ rearrangements found in the thymus [23, 24]. This suggests that there is not a strong selective pressure either for or against cells that carry trans-rearrangements that is based on the presence of the trans-rearrangement. Interestingly, as in the case of the ATM gene described above, the SCID gene product (the catalytic subunit of a DNA-dependent protein kinase [25, 26]) also carries a carboxy-terminal PI3-kinase motif. Thus, two inherited disorders in which a high incidence of lymphoma can be observed or induced, and in which the frequency of trans-rearrangements can become notably elevated, carry mutations in genes that share a motif believed to be involved in cellular proliferation and/or response to DNA damage.

Measurement of other trans-rearrangements

We have focused on the TCRγV-TCRβJ trans-rearrangement in our studies, but we have assayed other combinations of trans-rearrangements as well. In AT, we and others have found that many different combinations of trans-rearrangements are increased in frequency [7, 27]. In the SCID model system, TCRγV-TCRβJ rearrangements appear to be the most informative and correlative with lymphoma risk. In SCID the TCRδ locus in particular does not show a remarkably increased involvement in trans-rearrangements following radiation. TCRδ is relatively active in the genome of the unirradiated SCID animal. Following irradiation, genomic structural analysis of TCRδ is consistent with excision of this region from the chromosome. It is possible that the change in chromatin status and location brought about by this excision makes the involvement of this locus in trans-rearrangement much less likely. This is supportive of hypotheses that propose topographical and topological ‘accessibility’ as a necessary prerequisite to DNA rearrangement [28–31].

Other assays of genetic instability-corroborative data

Many of the populations studied with the assay of trans-rearrangement have been analyzed using measures of other types of genetic instability. These studies have substantiated the destabilizing effect of insecticides [32], chemotherapy [33–35], and radiation [36] for a variety of these different types of instability as well.

Conclusion

The absolute number of trans-rearrangements in thymus or peripheral blood correlates with risk of certain human or murine populations for the development of lymphoma. This correlation obtains whether the propensity is due to an inherited or acquired influence. Thus, an assay of trans-rearrangements may be capable of serving as a biomarker of lymphoma risk. The trans-rearrangements are apparently ‘innocent’ in this process, playing no direct role in the process of malignant transformation. As a corollary to this, an individual with a high number of such rearrangements should not be considered to have lymphoma at that moment, nor to absolutely be destined to develop lymphoma. The hypothesis would be that at the time that the level of trans-rearrangements is seen to be elevated there is a greater possibility for the occurrence of ‘malevolent’, frankly cancer-promoting translocations. Trans-rearrangements are not fortuitous biomarkers of lymphomagenesis because the basic mechanism that is responsible for their formation, V(D)J recombination, is, at least in part, the same mechanism responsible for the majority of chromosomal translocations in humans associated with the development of lymphoid malignancy [37].

References


