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**BRIEF REVIEWS**

**TCR trans-Rearrangements: Biological Significance in Antigen Recognition vs the Role as Lymphoma Biomarker**

Atef Allam* and Dieter Kabelitz†

**V(D)J rearrangements occur within loci of TCR and BCR genes, thus generating the diversity of the AgR repertoire. In addition, interlocus V(D)J rearrangements occur, giving rise to so-called “trans-rearrangements.” Such trans-rearrangements increase the diversity of the immune receptor repertoire and can be expressed as functional chimeric TCR proteins on the surface of T cells. Although chimeric receptors are not pathogenic per se, the frequency of AgR trans-rearrangements correlates with the level of genetic instability and thus could be used as a predictive biomarker for lymphoma risk. The Journal of Immunology, 2006, 176: 5707–5712.**

B cell receptor and TCR Ag-recognition polypeptide chains are encoded by sets of gene segments instead of single contiguous DNA sequences (1, 2). They consist of variable (V), diversity (D), and joining (J) gene clusters, and one gene out of each segment is selected to form V(D)J rearrangements (3). The recombination-activating proteins RAG1 and RAG2 orchestrate this process (4). Different B or T cells rearrange a different segment in each pool, thereby creating one level of AgR diversity. Moreover, the enzyme TdT incorporates nontemplate (N) nucleotides to the ends of V, D, and J gene segments before their joining (5). These inserted nongermline-encoded nucleotides at V(D)J junctions provide additional somatic diversification of the available germline repertoire. Thus, V(D)J recombination endows B and T lymphocytes with the capacity to specifically recognize an almost infinite array of Ags.

The mechanism of generation of TCR gene trans-rearrangements

The aforementioned V(D)J recombination occurs within the AgR loci (intralocus rearrangement). Strikingly, such rearrangements can also occur between two different loci (interlocus rearrangements or trans-rearrangements) (6–10). Aberrant interchromosomal V(D)J recombinations are physiologically prevented by several surveillance mechanisms (11, 12). Nevertheless, trans-rearrangements occasionally arise by mechanisms, including gene conversion and recombination and subsequent reintegration of excision products from prior cis-rearrangements (13). However, genetic evidence indicates that trans-rearrangements usually result from chromosomal translocation or inversion that occur at a very low frequency in TCR and even more rarely in BCR loci (6, 10, 13, 14). For example, V8-1y trans-rearrangements have been calculated to occur in PBL at a frequency of 1/200,000 (10). Human chromosome 7 carries the TCRβ genes at one end, whereas the TCRγ locus lies at the opposite end (Fig. 1). An inversion of this chromosome juxtaposes Vγ genes to β-chain gene segments (DβJβCB) at one chromosomal end and Vβ(DBJ) genes to JγCγ at the opposite end (6, 15). This results in a rearrangement of β genes with gene segments of the γ chain. Accordingly, an interlocus instead of intralocus rearrangement and hence a trans-rearrangement instead of a conventional rearrangement occurs (Fig. 1). Depending on the locus of inversion and re-ligation of the respective chromosome, various kinds of trans-rearranged TCR genes arise. Another example of such trans-rearrangements is an inversion of chromosome 14 in tumor cells of patients with childhood acute lymphoblastic leukemia of B cell lineage (7, 16, 17). This inversion creates a site-specific recombination event between an Ig H chain variable gene (IgVH) and the joining segment of a TCRα chain (TCRαJ). Consequently, this event results in the generation of a hybrid gene, partly Ig and partly TCR (IgVH-TCRα trans-rearrangement). Other types of hybrid transcripts were generated by splicing of an already rearranged TCRVγ-Jγ exon to the TCRβ constant region (trans-splicing) (6). Sequence analysis suggested that these trans-rearrangements arise through the action of normal lymphocyte recombinase, involve trans-recognition of heptamer/nonamer recombination signals, and follow the 12/23 spacer rule (10, 17). Additions of N nucleotides are frequently detected in trans-rearranged receptor genes (9, 10). These hybrid genes were transcribed into mRNA. Strikingly, sequence analysis of these mRNA showed that they generally contain a complete open reading frame and are translated into a chimeric protein displayed on the cell surface (8–10).

Known chromosomal abnormalities related to the trans-rearrangements are inversions involving chromosomes 7 and 14.

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mice homozygous for Mre11 and NBS1 hypomorphic alleles instabilities and predisposes to the development of lymphoid combination (31–34). A mutation in ATM causes chromosomal rearrangements. The rejoining of V(D)J-recombination intermediates, as well as the repair of double strand breaks, induced by exogenous DNA-damaging agents such as ionizing radiation occur mainly by nonhomologous end joining in G1 cells in vertebrates (25). A mutation of components required for nonhomologous end joining was found to increase the V(DJ) intergenic recombination (26). Thus, in mice lacking a functional DNA-protein kinase C, cells with a high level of unresolved coding ends and signal ends are generated (27, 28). In fact, Tevelev and Schatz (29) reported that recombination intermediates, if not joined or kept in a synaptic complex, could potentially be misjoined to any available ends. Moreover, secondary rearrangements (e.g., from Vγ to Jβ) were blocked in normal individuals but not in patients with ataxia telangiectasia (AT)2 (30), a disorder with increased rates of TCR trans-rearrangements (10, 15). AT patients have a homozygous mutation in the ataxia telangiectasia-mutated (ATM) gene, which codes for a serine-threonine protein kinase that phosphorylates Mre11 and NBS1 (Nijmegen breakage syndrome protein) and is required for the repair of DNA breaks and for V(DJ) recombination (31–34). A mutation in ATM causes chromosomal instabilities and predisposes to the development of lymphoid malignancy in AT patients and in ATM-deficient mice and mice homozygous for Mre11 and NBS1 hypomorphic alleles (35–38).

Increase in V(DJ) trans-rearrangements in pathological and pathophysiological conditions

TCR trans-rearrangements occur at a very low frequency (in the order of 1/200,000 PBL) in normal individuals (9, 10). However, 50- to 100-fold increased frequencies are observed in pathological conditions with higher chromosomal instabilities such as AT (10, 15) and lymphoid leukemias (7, 20). Intriguingly, the frequency of TCR trans-rearrangements can rapidly increase in some situations. Several fold increases in the frequency of trans-rearrangements were seen in patients undergoing chemotherapy for the treatment of lymphomas, leukemias, or solid tumors (39, 40). This increase was transient and returned to the basal level after cessation of chemotherapy. TCR trans-rearrangements have also been studied in mice models. In the SCID mouse, a marked increase in trans-rearrangements compared with BALB/c mice was observed (41, 42). The inability of SCID cells to promptly resolve their recombination ends seems to expose the ends to a random joining process and consequently to increase the trans-rearrangement levels (43–45). In line with similar observations in patients during radiation therapy, trans-rearrangement levels were also raised 50- to 100-fold by irradiation of mice (45).

The trans-rearrangement as a potential biomarker for the prediction of cancer

Genomic instabilities are the hallmark of most tumors and are believed to be a prerequisite for tumorigenesis (46). Beside the hereditary factor, external events such as exposure to hazardous chemicals or ionizing radiation may induce genomic instability (47, 48), which is correlated with the frequency of trans-rearrangements. The frequency of Vγ-Jβ trans-rearrangements was 1:30,000 in PBL, and in parallel to this, the inversion in chromosome 7 was ~1:10,000 in T cells (49, 50). Furthermore, in mice, the ratio of cis- to trans-rearrangements is 500-1000:1 in normal cells (45). However, in thymocytes from irradiated SCID mice, this ratio is increased to ~6:1 (51). Thus, the assessment of the frequency of trans-rearrangements provides a measure of genetic instability. Studies on a group of agricultural workers from southern Minnesota and northern Iowa (cancer victims’ area) indicated that the frequency of trans-rearrangements was correlated with the exposure to cancer-promoting chemicals such as herbicides, pesticides, and fungicides. In this group, a 15- to 30-fold increased frequency of trans-rearrangements was observed (52). Moreover, when newborn SCID mice were irradiated, all animals developed thymic lymphomas by 20 wk of age (51). Within 2 wk of irradiation, a 50- to 100-fold increase in TCR trans-rearrangements (notably Vγ-Cβ) was observed. In accordance with this study, several other studies in humans, including ours, have indicated that the rate of Vγ-Cβ trans-rearrangements is a suitable indicator for genetic instability (9, 51). Thus, it appears that in situations where prediction of cancer is high, TCR trans-rearrangements are increased in parallel with the occurrence of genomic abnormalities and a long time before the diagnosis of cancer is made. This suggests that the measurement of the frequency of trans-rearranged TCR genes in PBL might be a sensitive assay to

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2 Abbreviations used in this paper: AT, ataxia telangiectasia; ATM, ataxia telangiectasia mutated; DP, double positive.
determine the predisposition to cancer if other reasons for transient increases, such as ongoing chemotherapy, exposure to environmental agents, or irradiation, are excluded (51).

*Cell surface expressed chimeric TCR are functional*

Sequence analysis of trans-rearranged transcripts showed that they generally maintained a correct open reading frame at the V-J junction, and the hybrid V-J exon was correctly spliced to a TCR constant region, thus allowing translation into a functional AgR chain (8–10). This hybrid chain was able to pair with intralocally rearranged TCR chains. For example, the hybrid TCR Vγ-Cβ chains were paired to TCR Vα-Cα chains (8). Trans-rearranged chimeric TCR still recognize antigenic peptides within the MHC grooves and are functionally similar to classically rearranged receptors. Cell lines with different types of trans-rearranged TCR have been generated (8). Functional analysis of such T cell lines revealed strong proliferative or cytotoxic responses toward allogeneic cells (Fig. 2). Together with the presence of CD3 and either CD4 or CD8 coreceptors on most of the T cell clones (8, 9), these results suggest that ligands recognized by trans-rearranged TCR are very similar to those recognized by conventional T cells. In addition to previously defined Vγl family-Cβ trans-rearrangements (8), we identified and characterized Vγ-Cβ trans-rearrangements involving the most frequent Vγ gene in human peripheral blood, i.e., Vγ9 (the single VγII family member) (9). T cells carrying the chimeric Vγ9-Cβ receptor chains responded to stimulation with mAb against Vγ9 and Cβ by proliferation and secretion of TNF-α and IFN-γ. While Meydan et al. (53) observed a clonal expansion of such T cells carrying trans-rearranged TCR, our own lines were oligo/polyclonal in nature based on PCR and flow cytometric analysis (8, 9). Given that alloreactivity is a proven characteristic of αβ and not of γδ T cells, expressing trans-rearranged TCR are not only phenotypically but also functionally similar to αβ T cells. The observed alloreactivity (8) indicates that conventionally rearranged and trans-rearranged TCR induce similar cellular responses and presumably similar signaling pathways. There are several implications with respect to the process of TCR selection and the structural relationship between αβ and γδ TCR. Because most γδ T cells are CD4−CD8− and are not alloreactive, this would indicate that the same Vγ gene could participate in the recognition of distinct sets of ligands depending on the TCR chain with which it associates. Furthermore, the trans-rearrangement model suggests a close structural relationship between Vγ and VB regions, an assumption that is also supported by the fact that both V regions display superantigen reactivity (54, 55), unlike Vα and VB regions. Finally, these studies suggest that TCR alloreactivity is determined by the repertoire selection process operating during lymphocyte development rather than by structural features specific to Vα/Vβ regions.

*A survey of described trans-rearranged TCR genes*

While many kinds of trans-rearrangements are detected, trans-rearrangements between the TCRγ and TCRβ loci are the most abundant (45). Early studies reported that both Vγ-Jγ and Dβ-Jβ occur simultaneously (23, 25), which gives rise to more opportunities for this kind of trans-rearrangement to take place. Table I lists the described TCR trans-rearrangements.

**Immunological consequences of TCR trans-rearrangements**

1) **Trans-rearrangements as a possible compensation mechanism.** Strikingly, the frequency of trans-rearrangements increases in pathological situations, exhibiting a marked deficiency in lymphocytes number and/or function (9). For instance, while AT patients have a skewed TCR repertoire and a marked decrease in thymic output (56), a high frequency of TCR trans-rearrangements has been observed (6, 9, 15). Furthermore, TCR trans-rearrangements were raised under stressful conditions such as exposure to irradiation or to hazardous chemicals where immune system dysfunctions were observed (47, 48, 52). Taken together, this raises the possibility that the

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**FIGURE 2.** T cells harboring TCR trans-rearrangements can express functional chimeric TCR proteins on the cell surface. *Left,* Conventional αβ TCR with β-chain consisting of rearranged VβDβJβCβ genes. *Right,* chimeric αβ TCR with β-chain consisting of Vγ rearranged to DβJβCβ. T cells expressing chimeric TCR respond to antigenic or anti-TCR mAb stimulation like conventional T cells with cytotoxic effector activity, cytokine production, and proliferation.

Target cell

MHCI-Ag peptide

CD8 coreceptor

CD8 T cell with rearranged TCR

TNFα and IFNγ secretion

Proliferation
increased occurrence of (functional) trans-rearranged receptors helps to broaden the TCR repertoire in situations of skewed immune responses. However, it remains to be determined how these cells function in vivo. Functional assays such as an adoptive transfer into SCID- or Rag2-deficient mice might help to elucidate this point.

2) Cells expressing trans-rearranged TCR may undergo negative selection. The intrathymic differentiation differs in developing αβ and γδ T cells. While γδ T cells remain mainly CD8−CD4+, αβ T cells develop from CD8+CD4+ double-positive (DP) intermediates. Thus, it is possible that cells with trans-rearranged TCR are generated from CD8+CD4+ DP thymocytes. Consequently, they would be examined by self-peptide-MHC complexes to become committed to either CD8 or CD4 single-positive T cells. The fact that CD4 and CD8 expression is induced after either Vβ8Jβ8BCβ or VγJβBCβ rearrangement within the precursor cell would indicate that transition to the DP stage is primarily dictated by the locus of origin of the constant rather than the variable part of the rearranged gene, which is in accordance with earlier observations made by others (57). Cells with trans-rearranged TCR show a distinctive distribution in the mouse depending on the type of trans-rearrangement. T cells with trans-rearranged VγJγ, Vδ-Jγ, and Vγ-Jβ are present in the thymus and considerably less in other organs, whereas V8-Jβ and IgVH-Jβ are detected only in the thymus (10). This pattern of distribution indicates that these cells undergo a negative selection in thymus. Potential reasons for the negative selection might include the conformation of the trans-rearranged receptor, rendering it incapable of detecting the architecture of self-MHC-bound self-peptide. Consequently, such receptors would be unable to receive a life signal and therefore would undergo death by neglect. Another possibility is that these cells undergo apoptosis since thymic output is extremely low in patients with a high frequency of trans-rearrangements, as well as in SCID mice (58, 59).

3) Trans-rearrangements increase the diversity of the immune receptor repertoire. Since a small yet significant fraction of PBL express trans-rearranged TCR chains on their surface in all individuals (8, 9), it is obvious that trans-rearrangements contribute to the combinatorial diversification of the immune system repertoire. Sequence analysis of Vγ chains shows that the joining of a Vγ gene segment to a D gene segment is unusual in trans-rearranged TCR chains. This further illustrates the role of trans-rearrangements in increasing the diversity of the immune receptor repertoire. Furthermore, sequence analysis of Vγ-Jβ trans-rearrangements showed many variations in D gene segment assembly. While in cis-rearrangements a rearranged D gene segment was flanked by N regions, in some trans-rearranged receptors only one N region nucleotide was added (Table I). Remarkably, Tycko et al. (23) observed constraints on recombination within the TCR locus that prevented Vγ-Jγ2 rearrangements while allowing rearrangements in trans. Taken together, the mechanism of trans-rearrangement adds more diversity to the AgR repertoire.

4) The trans-rearrangement paradigm is in accordance with the αβ and γδ TCR gene rearrangement model. The commitment to the αβ or γδ lineage in the thymus is not fully

### Table I. A survey of reported TCR trans-rearrangements

<table>
<thead>
<tr>
<th>V</th>
<th>N</th>
<th>(D)</th>
<th>N</th>
<th>J</th>
<th>trans-Rearranged Receptor Chain References</th>
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</thead>
<tbody>
<tr>
<td>TGGGAAAG</td>
<td>GACGTA</td>
<td>GGAAGCT</td>
<td>ACAGATA</td>
<td>Vβ1 Jβ2.1 Jβ2.3</td>
<td>(10)</td>
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<tr>
<td>ACTGCTGCCTGGGAG</td>
<td>ACCAGTCTCAGCTGGGTG</td>
<td>TTTTG</td>
<td>ACAGATTAATACTCCTCT</td>
<td>Vγ2 D8 Jγ2</td>
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<td>GCACTGACTGACTG</td>
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a These trans-rearrangements are provided in amino acid sequences only.
understood (60). One model postulates that γ and δ loci are fully rearranged, whereas at the same time the β locus is partially rearranged (D-J). If the γ and δ genes are not successfully rearranged, the rearrangement of β will continue, and the α chain will start to rearrange (61). The trans-rearrangement phenomenon sheds some light on which kind of genes are simultaneously available for the rearrangements. While γ, δ, and β hybrids are found, Vα trans-rearrangements have as yet not been detected. Remarkably, Jo gene segments have been detected in trans-rearranged receptors (10). Together, the trans-rearrangement study shows that precisely the Vα and not the entire α gene regions are still not available to rearrange at the time when the rest of TCR gene segments are. We hypothesize that a specific mechanism is required to open the chromatin region of Vα, an assumption that requires further investigation.

**Concluding remarks**

Many studies showed that rearrangements can occur between different genes of TCR and BCR, thereby generating V(DJ) trans-rearrangements. Such trans-rearrangements increase the diversity of the immune receptor repertoire. The trans-rearranged chimeric TCR displayed on the cell surface exert alloreactivity and respond to selective stimuli by cytokine production. Furthermore, trans-rearrangements are potentially “benevolent” errors in the immune system and might serve as a good marker for genomic instability. 

**Disclosures**

The authors have no financial conflict of interest.

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