Research Article

The Feature of Distribution and Clonality of TCR γ/δ Subfamilies T Cells in Patients with B-Cell Non-Hodgkin Lymphoma

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Received 30 March 2014; Accepted 3 May 2014; Published 21 May 2014

1. Introduction

B-cell non-Hodgkin lymphoma (B-NHL) is a heterogeneous group of malignant lymphoproliferative disorders originating in B lymphocytes, which comprises approximately 80–85% of newly diagnosed cases with NHL. Although current therapeutic strategies, including standard chemotherapy, unlabeled or radiolabeled monoclonal antibodies, high-dose chemotherapy following autologous peripheral blood stem cell transplantation, or allogeneic hematopoietic stem cell transplantation, have significantly improved the outcome of this disease, the majority of patients relapse or become resistant to prior therapies. Therefore, novel strategies, such as cellular immunotherapy, are increasingly investigated [1].

Poor cellular immune function may relate to carcinogenic processes and to worse prognosis in tumor patients. Moreover, the progression of tumor might further induce the cellular immune suppression. In recent years, molecular analysis of the T-cell receptor (TCR) utilization feature, based on the principle of TCR α, β, γ, and δ gene rearrangement and deletion rearrangement, has proven to be an effective technique for studying the distribution of T cell repertoire, the diversity of TCR subfamilies [2, 3], the antigen specific expansion of T-cell clones, and the recent thymic output...
function [4, 5]. This in turn can help to characterize the feature of host T-cell immune status and the identification of T-cell populations of interest in cancer, as well as the peripheral immune repertoire reconstitution after hematopoietic stem cell transplantation (HSCT).

T cells possessing a γδ TCR are a small subset of human T cells (1–10% of all peripheral blood T cells). These cells share effector functions with αβ T cells as well as with natural killer (NK) cells, particularly the capacity to interact with dendritic cells (DCs) [6, 7]. Mice deficient in γδ T cells show a significantly increased incidence of tumors and provide clear evidence for an immune surveillance function of these unconventional lymphocytes [8]. Human Vγ9Vδ2 T cells can kill a broad spectrum of tumor cells with or without reduced MHC class I molecules expression in an MHC-unrestricted manner [9–14]. Moreover, γδ T cells can migrate as infiltrating lymphocytes into solid tumors [9] and can recognize and eliminate cultured malignant cells (primary cells or cell lines) from myeloma [10, 11], non-Hodgkin lymphoma [12], prostate cancer [13], renal cell carcinoma [14], colon carcinoma [15], and squamous cell carcinoma [16]. Obviously, γδ T cells play an important role in immunosurveillance and antitumor response and become more and more attractive for cell therapy strategies against cancer. However, little is known about γδ T-cell immune status in B-NHL patients. Bartkowiak et al. have reported that the high restricted TCR Vγ subfamily usage that is predominant for TCR VγII (Vγ9) was characterized in chronic lymphocytic leukemia (CLL) [17].

T cells recognize specific ligands by specific TCRs, which are glycoprotein heterodimers comprising either α/β or γ/δ chains. Rearrangement of the individual variable (V), diversity (D), joining (J), and constant (C) regions leads to the creation of the hypervariable complementarity determining region 3 (CDR3) of the functional TCR, which plays a pivotal role in the recognition of antigenic epitopes [18, 19]. γδ T cells rearrange and express clonally diverse receptors in a manner similar to αβ T lymphocytes; however, the V, D, and J element repertoire in the TCR γ and TCR δ loci is limited in number. The TCR γ gene contains at least 14 functional variable (TCR Vγ) segments belonging to four subgroups (i.e., TCR VγI to IV), and the TCR δ gene contains at least eight functional TCR Vδ segments that are subdivided into eight Vδ subfamilies (i.e., Vδ1–Vδ8). Our previous study showed that restricted TCR Vα and Vβ repertoire expression and clonal expansion of αβ T cells were observed in peripheral blood from patients with diffuse large B-cell lymphoma (DLBCL) [20]. To further characterize the γδ T-cell immune status in B-NHL, we investigated the distribution and clonality of TCR Vγ and Vδ repertoire in peripheral blood (PB), bone marrow (BM), and lymph node (LN) from patients with B-NHL.

2. Materials and Methods

2.1. Samples. Four male patients with B-NHL diagnosed according to the World Health Organization (WHO) criteria were enrolled in the present study (designated as C1–C4).

The clinical characteristics of these patients are described in Table 1. After the patient’s consent, PB samples were obtained from all of the four patients, BM samples were obtained from 3 (C1, C2, and C3) of the four patients, and lymphoma-infiltrated LN samples were obtained from diagnosed biopsy in 3 patients (C1, C2, and C4), respectively. All procedures were conducted according to the guidelines of the medical ethics committees of the Health Bureau of Guangdong Province, China.

2.2. Mononuclear Cells Isolation, RNA Isolation, and cDNA Synthesis. Mononuclear cells of PB or BM samples (PBMCs or BMMCs) were isolated by Ficoll-Hypaque gradient centrifugation. RNA was extracted from PBMCs, BMMCs, or LN homogenate using a RNA extraction buffer according to the manufacturer’s protocol (Trizol, Invitrogen, USA). The RNA quality was analyzed in 0.8% agarose gel stained with ethidium bromide. Two μg RNA was reversely transcribed into the first single-strand cDNA with random hexamer primers, using reverse transcriptase, Superscript II Kit (Gibco, USA). The cDNA quality was confirmed by RT-PCR for β2 microglobulin gene amplification.

2.3. RT-PCR for the TCR Vγ and TCR Vδ Subfamily Amplification. Three sense TCR Vγ primers and a single TCR Cy reverse primer or eight TCR Vδ sense primers and a single TCR Cδ primer were used in unlabeled PCR for amplification of the TCR Vγ and Vδ subfamilies, respectively. Subsequently, a run-off PCR was performed with fluorescent primers labeled at 5’ end with the FAM fluorophore (Cy-FAM or Cδ-FAM) (TIB MOLBIOL GmbH, Berlin, Germany). The sequences of primers are listed in Table 2. The PCR was performed as previously described [21]. Aliquots of the cDNA (1 μL) were amplified in 20 μL mixtures with one of the three Vγ primers and a Cy primer or one of the eight Vδ primers and a Cδ primer. The final reaction mixture contained 0.5 μM of the sense and antisense primers, 0.1 mM dNTPs, 1.5 mM MgCl₂, 1 × PCR buffer, and 1.25 U Taq polymerase (Promega, USA). The amplification was performed in a DNA thermal cycler (BioMetra, Germany) with 3 min denaturation at 94°C and 40 PCR cycles. Each cycle consisted of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, respectively, and a final 7 min elongation at 72°C. The PCR products were stored at 4°C and ready for genescan analysis.

| Table 1: Clinical characteristics of the included patients with B-NHL. |
|-----------------|-------|------|---------|--------|
| Patient number | Gender | Age (yrs) | Disease subtype | Ann Arbor staging |
| C1              | Male   | 57    | DLBCL   | IVA    |
| C2              | Male   | 60    | DLBCL   | IVB    |
| C3              | Male   | 56    | DLBCL   | IVA    |
| C4              | Male   | 76    | SLL     | IIIB   |

DLBCL: diffuse large B-cell lymphoma; SLL: small lymphocytic lymphoma; yrs: years.
2.4. Genescan Analysis for TCR V\gamma and V\delta Subfamily Clonality. Aliquots of the unlabeled PCR products (2 \muL) were subjected to a cycle of run-off reaction with fluorophore-labeled Cy-FAM or C\delta-FAM primer, respectively. The labeled runoff PCR products (2 \muL) were heat denatured at 94 \degree C for 4 min with 9.5 \muL formamide (Hi-Di Formamide, ABI, USA) and 0.5 \muL of size standards (Genscan-500-LIZ, Perkin Elmer, ABI). The samples were then loaded into a 3100 POP-4 gel (Performance Optimized Polymer-4, ABI, USA) and resolved by electrophoresis in a 3100 DNA sequencer (ABI, Perkin Elmer) for size and fluorescence intensity determination using Genescan software [21]. Since the positions of the V\gamma/V\delta and Cy/C\delta primers are fixed, the length distribution observed in the PCR V\gamma-Cy/V\delta-C\delta products depends only on the size of the rearrangement of V-J (in TCR\gamma) or V-D, D-J (in TCR\delta) gene segment and the randomly inserted nucleotides (VN(DN)). After electrophoresis on an automated sequencer and subsequent computer analysis, the products of different size could be separated and expressed as different peaks.

3. Results

3.1. The Distribution and Clonally Expanded TCR V\gamma and V\delta T Cells in Peripheral Blood from Patients with B-NHL. The expression of TCR V\gamma and V\delta subfamilies was detected by RT-PCR, and no PCR products were scored as negative for the corresponding TCR subfamily by agarose gel electrophoresis. Only one or two of the three TCR V\gamma subfamilies and four to six of the eight TCR V\delta subfamily members could be detected in PB samples. V\gammaI, V\deltaI, and V\deltaII were expressed in all samples, whereas V\gammaII expression was absent in all of the four samples. In addition, V\gammaIII (3/4), V\deltaIII (2/4), V\deltaIV (1/4), V\deltaV (1/4), V\deltaVI (2/4), V\deltaVII (2/4), and V\deltaVIII (2/4) could be detected in partial cases (Figure 1).

The clonality of TCR V\gamma and V\delta subfamily T cells was identified by Genescan analysis, which was used to identify the CDR3 size and assess the spectratype pattern visually. Polyclonality of TCR V\gamma and V\delta subfamily T cells displays a Gaussian or nearly Gaussian-like distribution consisting of multiple peaks (usually 6–8), and any deviation from the Gaussian profile (skewed repertoire) would indicate clonally expanded pattern. Oligoclonality and biclonality shows a skewed spectratype profile with a single dominant peak and double peaks, respectively. Oligoclonal trending is between polyclonal and oligoconal. The PCR product analysis produces only one peak, which means that CDR3 lengths are identical, named as the monoclonal pattern [21, 22]. For the TCR V\gamma and V\delta subfamily clonality analysis, only the clonally expanded V\gammaIII subfamily was detected in samples from C2 and C4 cases, whereas clonally expanded V\delta T cells could be identified in almost all subfamilies (except for V\deltaI) in different patients. Additional, at least one clonally expanded TCR V\delta subfamily member was found in PB in every patient. The distribution of the clonally expanded \gamma T cells in PB is shown in Figures 1 and 2.

3.2. Different Expression Patterns of TCR V\gamma and V\delta in Peripheral Blood, Bone Marrow, or Lymph Node. The expressions of V\gammaI/V\deltaIV/V\gammaVIII/V\deltaVII/V\deltaVIII and V\gammaII/V\deltaV were absent in BM and LN samples, respectively. Clonal expansion of TCR V\delta repertoire could be found in some TCR V\delta subfamilies in PB, BM, or LN, which displayed different patterns in different patients (Figure 3). In patient C1, the clonally expanded V\delta2, V\delta5, V\delta6, and V\delta8 subfamily T cells could be found in PB, whereas only the clonally expanded V\delta2 could be detected in BM, and there has been no clonal expansion of TCR V\gamma/V\delta subfamilies in LN. In patient C2, the clonality of V\delta4, V\delta6, and V\delta8 changed from oligoclonality or oligoconal trend in PB to polyclonality in LN, whereas the expression of those was absent in BM. In patient C3, the clonally expanded V\delta2/V\delta3 and V\delta1/V\delta2 T cells were found in PB and BM, respectively. In patient C4 with SLL, the clonally expanded V\delta3 and V\delta2/V\delta3 T cells were found in PB and LN, respectively. Interestingly, the oligoclonally expanded V\delta3 T cells could be found in PB from patients C3 and C4 and in LN from patient C4.

4. Discussion

Analysis of alterations in the TCR repertoire is an effective investigational approach that may help to understand involved immunological abnormalities and provide guidance for clinical applications using this information [23, 24]. Recent data indicated that T-cell immunodeficiency is a common feature in different hematological malignancies, including the absence of TCR \alpha and \gamma\beta subfamilies, decreased diversity of TCR repertoires, reduced thymic recent output function (naïve T cells), and lower frequencies of TCR subfamily naïve T cells [25]. Apart from \alpha\beta T cells, \gamma\delta T cells also play important roles in immunosurveillance and anticaner response. Different TCR V\gamma and V\delta subfamily expression patterns have been reported in patients with leukemia, myelodysplastic syndrome (MDS), and immune thrombocytopenic purpura (ITP) [3, 17, 26, 27]. However,
little is known about the distribution and clonality of the TCR Vγ and Vδ subfamilies in B-cell lymphoma. In the present study, we analyzed the distribution and clonal expansion of TCR Vγ and Vδ T cells in four B-NHL patients and compared the different expression patterns of TCR Vγ and Vδ in peripheral blood, bone marrow, or lymph node in individual cases. In contrast to healthy individuals and patients with MDS or ITP previously reported [26, 27], the absence of TCR VγII subfamily was found in all blood, marrow, and lymph node samples from B-NHL patients, which imply a widespread restricted TCR Vγ repertoire expression pattern may be a feature in patients with B-NHL. Moreover, the distribution of Vγ and Vδ subfamilies was not identical in samples between peripheral blood, bone marrow, or lymph node, and this may be due to the distribution or expansion of γδ T cells in different immune organs, however, further investigation is needed to summarize this feature in a large cohort samples and follow up the change of γδ T-cell repertoire on the outcome of the patients.

Like the change of clonality of TCR subfamily in leukemia [17, 27], clonally expanded TCR Vδ subfamilies could be found in peripheral blood from all of four B-NHL patients, which is thought to be related to the tumor associated antigens [17, 20, 27]. In this study, we analyzed the distribution of clonally expanded Vδ T cells not only in peripheral blood, but also in bone marrow or lymph node, we were interested to find out the identical expanded Vδ T cell clones, similar clonal expanded Vδ2 subfamily T cells were detected in both peripheral blood and bone marrow samples in two cases with DLBCL, and similar clonality of Vδ3 subfamily T cells was identified in peripheral blood and lymph node in one case with SLL; these preliminary data suggested that these Vδ T-cell clones might respond to the same B-cell lymphoma-associated antigens. However,
differently reactive clonally expanded Vδ T cells between peripheral blood, bone marrow, and lymph node may be due to local immune response. Further investigation is needed to determine whether these clonally expanded T cells are related to antilymphoma cells.

In addition, in contrast to the clonally expanded Vδ2, Vδ3, and Vδ4 T cells in lymph node from the SLL patient (C4), none clonal expansion of TCR Vγ or Vδ T cells was detected in lymphoma cell-infiltrated lymph node samples donated by the two cases with DLBCL (C1 and C2), suggesting that the deficiency of clonal expansion of γδ T cells in lymphoma cell-infiltrated lymph node may be another feature in DLBCL. However, whether it is related to tumor microenvironments of lymph node remains an open question and needs to further explore.

5. Conclusion

In this study, we characterized the distribution and clonality of Vγ and Vδ repertoire in peripheral blood, bone marrow, and lymph node from B-NHL patients, we found obviously different features of restrictive usage and clonal proliferation of TCR Vγ and Vδ subfamilies in individual patients as well as in different immune organs; even if we found some identical clonally expanded Vδ subfamily T cells in peripheral blood and bone marrow, similar clonal expanded Vδ subfamily T cells in peripheral blood and bone marrow may be related to the same B-cell lymphoma-associated antigens, while the different reactive clonally expanded Vγ/Vδ T cells may be due to local immune response. However, whether it is related to different antigen stimulation and tumor microenvironments remains an open question and needs to further explore.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This study was supported by Grants from the National Natural Science Foundation of China (no. 81270604),
the Guangdong Science & Technology Project (no. 2012B050600023), the Fundamental Research Funds for the Central Universities (no. 21614308), and the Medical Science Research Foundation of Guangdong Province (no. B2012195).

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