Selective Gamma-Chain T-Cell Receptor Gene Rearrangements in a Patient with Omenn’s Syndrome: Absence of V-II Subgroup (Vγ9) Transcripts

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Received 8 April 1996/Returned for modification 2 May 1996/Accepted 19 June 1996

Only γ-chain T-cell receptor transcripts utilizing V-I subgroup gene segments were found in peripheral blood lymphocytes from a patient with Omenn’s syndrome. γ-Chain T-cell receptor transcripts utilizing the Vγ9 (V-II subgroup) gene segment were absent in peripheral blood lymphocytes from this patient. Vγ9-J1.2 Cα1 rearrangements are those primarily found in peripheral blood lymphocytes (70 to 85%) from normal donors.

Omenn’s syndrome is an autosomal recessive combined immunodeficiency with hyperesinophilia (19), presenting early in life with widespread erythroderma, protracted diarrhea, hepatosplenomegaly, lymphadenopathy, and failure to thrive (19). There is severe lymphocyte depletion in the thymus and lymph nodes, in contrast to a high number of poorly functional T cells in the peripheral blood as well as infiltrating the skin, gut, liver, and spleen (7, 19, 23). It has been suggested that the basis of this syndrome is graft versus host disease caused by engraftment of maternal lymphocytes; however, no evidence supporting this hypothesis has been found (7, 23, 28). The syndrome appears to represent an intrinsic lymphocytic lineage defect, and it is curable by bone marrow transplantation (9).

The γδ T-cell receptor (TCR) utilizes two different constant region gene segments, the Cγ1 and the Cδ2 (2, 5, 10, 11). γδ TCRs employing the Cγ1 gene segment are disulfide-linked, whereas those employing the Cδ2 segment are non-disulfide-linked (2, 5, 10, 11). Due to their genomic organization, Jγ1 (1.1, 1.2, and 1.3) gene segments exclusively rearrange to the Cγ1 gene segment and Jδ2 (2.1, 2.3) gene segments exclusively rearrange to the Cδ2 gene segment (reviewed in reference 1). We have shown (13) that in the peripheral blood of normal individuals, V-I subgroup human γ-chain TCR gene segments preferentially rearranged with Jγ2.1 Cγ2 or Jγ2.3 Cγ2 gene segments. In contrast, >85% of the γ-chain transcripts in the peripheral blood that utilize the V-II subgroup (Vγ9) gene segment exclusively rearranged to Jγ2.1 Cγ1 gene segments (26).

We report here that peripheral blood lymphocytes from a patient with Omenn’s syndrome exhibited a restricted pattern of rearrangements of the γ-chain TCR genes. We amplified by PCR γ-chain cDNAs from the peripheral blood lymphocytes from this patient and cloned and sequenced the amplified cDNAs. Exclusive utilization of V-I subgroup gene segments was observed, and these were rearranged to Jγ2.1 Cγ2 gene segments. cDNAs utilizing the Vγ9 Jγ1 (1.1, 1.2, and 1.3) Cγ1 gene segments were not observed in the peripheral blood lymphocytes of this patient. Vγ9-J1.2 Cα1 rearrangements are those used primarily (70 to 85%) by peripheral blood lymphocytes in normal donors (26).

These findings were presented in part at the 1991 Annual Meeting of the Federation of American Societies for Experimental Biology, Atlanta, Ga. [9a].

Heparinized peripheral blood was obtained from a 2-month-old Caucasian female infant, who was admitted to the All Children’s Hospital and diagnosed with Omenn’s syndrome. The patient exhibited generalized desquamative rash since birth and generalized lymphadenopathy and massive splenomegaly of unknown etiology. A skin biopsy showed spongiosis with scattered necrotic keratinocytes, a finding consistent with the diagnosis of Omenn’s syndrome.

Significant T-cell lymphocytosis was evident in the peripheral blood. Over the second month of the life of the patient, leukocytes were consistently significantly elevated (50,000 to 79,800 cells per mm³; range, 4 determinations), and lymphocytes accounted for 56 to 80% of leukocytes. Moderately high levels of eosinophils were consistently observed. Representative results of a differential count were as follows: leukocytes, 50,000 per mm³; 4% segmented; 1% band; 79% lymphocytes; 1% monocytes; and 9% eosinophils. Flow cytometry analysis of mononuclear cells was carried out by standard methods using a battery of monoclonal antibodies. The following results were obtained (Leukocyte and lymphocyte counts were 79,800 cells per mm³ and 63,814 cells per mm³, respectively): CD2, 99%; CD3, 93% (59,371 cells per mm³); CD4, 37%; CD8, 56%; CD4/CD8, 0.7; CD5, 93%; CD7, 81%; and CD16, 7%. The proportion of B cells was reduced (to 1%) (kappa, 2%; lambda, 0%); however, their absolute numbers were normal for the age of the patient (638 cells per mm³). Similar results were obtained in an additional determination at a different time. A lymph node biopsy demonstrated abnormal lymph...
node architecture. The lymph nodes exhibited a granulomatous appearance, and large numbers of lymphocytes were present and were predominantly CD8 cells. Specifically, flow cytometry analysis of lymph node lymphocytes revealed the presence of the following populations: CD3, 76%; CD2, 76%; CD4, 12%; CD8, 52%; CD20, 12%; CD19, 0%; CD10, 0%; and CD33, 20%. Kappa and lambda chain-positive cells were not detected. Increased proportions of cells of the monocyte-macrophage lineage (25%) were also present. The combined proportion of CD4\(^+\) and CD8\(^+\) cells (64%) was lower than that of CD3\(^+\) cells (76%), in agreement with reports in the literature (8, 28, 29) that double-negative (DN) T cells (CD3\(^-\)CD4\(^-\)CD8\(^-\)) are increased in patients with Omenn’s syndrome. A bone marrow biopsy and aspiration revealed mixed cellular populations with primary hemopoietic cells and adequate and progressive maturation of cell lines (leukocytes, 3,797 cells per mm\(^3\)). An myeloid-to-erythroid ratio of 1.3 was found. Lymphocytes were present in increased numbers and comprised approximately 15 to 20% of the nucleated marrow cells. Atypical features were not observed in the lymphocytes or macrophages. The proportions of blast cells, monocytes, or histiocytes fell within the normal ranges.

Mononuclear cells were isolated by centrifugation on a Ficoll-Hypaque density cushion, as previously described (20), from heparinized peripheral blood from normal donors or the patient with Omenn’s syndrome. The mononuclear cells were passed through a nylon wool column (12), and the nonadherent cells were isolated and treated with OKT4 (anti-CD4) and anti-Leu11 (anti-CD16) monoclonal antibodies plus rabbit complement, as previously described (20). CD4\(^+\) and CD16\(^-\) cells were used for isolation of total RNA, as described previously (13). \(\gamma\)-Chain TCR-specific first-strand cDNA was synthesized by using a C\(_\gamma\)-specific primer (5’-TGTGAGCTGCAGCAGTAGAGTAT-3’), and the cDNA was amplified by PCR in a Thermocycler (Perkin-Elmer Cetus), as previously described (13). The following amplification steps were carried out: denaturation for 1 min at 95°C, annealing for 2 min at 55°C, and extension for 3 min at 72°C. A set of primers specific for the variable and constant gene segments were used as previously described (13). The V region (5’-end) primers were specific for the V-I subgroup (5’-TGCGGTGGGCCCTACTGGTGCTT-3’)(V\(_{\gamma}^g\)2, V\(_{\gamma}^g\)3, V\(_{\gamma}^g\)4, and V\(_{\gamma}^g\)5) or the V-II subgroup (5’-TGCTGACTGCTCCACACATC-3’)(V\(_{\gamma}^g\)9). The following C\(_\gamma\) primer was used as the 3’-end amplification primer: 5’-CCATGAGTACATGCTTTCAGGAGACATCTTCATCAG-3’. The amplified cDNAs were purified by Centricon-100 centrifugation (Amicon, Danvers, Mass.) and cloned into the pUC18 vector, and the recombinant plasmids were used to transform DH5\(_\alpha\) E. coli cells, as previously described (13). White bacterial colonies were screened by filter hybridization by using a C\(_\gamma\)-specific screening probe (5’-GGAAGGAAAATAGTGGGGTTTGGG-3’), as previously described (13). This probe is specific for both C\(_\gamma\)1 and C\(_\gamma\)2 gene segments, and it is located 5’ to the C\(_\gamma\) amplification primer. Thirteen clones were sequenced (Fig. 1) by the Sanger dideox method, as previously described (13). None of these clones were found in the GenBank database. All

<table>
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FIG. 1. Gamma-chain TCR transcripts from the peripheral blood of a patient with Omenn’s syndrome utilize J\(_{2\gamma}^g\)2\(_{2\gamma}^g\) gene rearrangements. Deduced amino acid sequences, shown above the nucleic acid sequences, are underlined for the V and J segments and are in italics for the N regions. Single-base substitutions are in parentheses. * out of frame or stop codon.
clones utilized V1-3, and three utilized V1-4. Furthermore, both V1-3 and V1-4 gene segments rearranged to J1-2 C1-2 segments. CDNAs utilizing the V9 J1-1 (11,1,2, and 1.3) C1-1 gene segments were not observed in the peripheral blood lymphocytes of this patient. This pattern of rearrangements is in contrast to rearrangements in peripheral blood lymphocytes of normal individuals, which preferentially use V9 (V-II subgroup) rearrangements to J1-1 C1-1 gene segments (26). We examined the expression of the V9 (V-II) gene segment in peripheral blood CDNAs amplified by PCR in three normal individuals (13). Under identical experimental conditions, including PCR primer concentrations, we identified a significant number of V9 clones (13). All the sequenced V9 clones exhibited rearrangements to J1-1 C1-1 gene segments. Conclusions regarding the exact level of expression of the different V1 segments (V1 versus V9) cannot be drawn, since our experiments are not quantitative. However, the absolute lack of V9 clones under identical experimental conditions of amplification and cloning is significant.

de Saint-Basile et al. (8) demonstrated that all γδ TCR+ T lymphocytes in a patient with Omenn’s syndrome were labelled by an anti-V1-1,4 monoclonal antibody (δTCS1) (29), while anti-V9 and anti-V2 did not label the γδ TCR + T cells. Although the anti-δTCS1 monoclonal antibody reacts with both disulfide-linked and non-disulfide-linked γδ TCR + T-cell clones (18, 21, 24), the vast majority of peripheral blood δTCS1+ T lymphocytes express non-disulfide-linked γδ TCR (4). These reported findings (8) are in agreement with ours. This particular patient (8) expressed increased proportions (determined by two-color flow cytometry) of γδ TCR + T cells. In addition, de Saint-Basille et al. (8) investigated the TCR genomic DNA rearrangements of peripheral blood or tissue lymphocytes by Southern blotting, using appropriate Cδ, Jδ, and Jγ probes. Different non-germline β-, γ-, and δ-chain TCR gene rearrangements were observed in these patients. The degree of oligoclonality of these non-germline rearrangements varied among different patients, and their functional significance is unknown.

A common feature of the preferentially expanded γδ TCR + or ωβ TCR + T cells in Omenn’s syndrome appears to be that they comprise DN T cells (CD3+ CD4− CD8−) (8, 28, 29). Preferential expansion of oligoclonal VB14+ DN T cells may account for the abnormal expression of certain cytokines (14). Interleukin-4 (IL-4) upregulates, while IL-2 and gamma interferon downregulate, immunoglobulin E production (15, 22). IL-5 enhances eosinophil maturation and degranulation (6). Melamed et al. (14) described the expansion of a DN T-cell population preferentially utilizing the VB14 gene segment in a patient with Omenn’s syndrome. These T cells expressed increased levels of IL-5 and decreased levels of IL-2 and gamma interferon, suggesting an association between these expanded DN T cells and the observed cytokine pattern.

Our results as well as those of de Saint-Basille et al. (8) suggest that peripheral blood T lymphocytes from patients with Omenn’s syndrome do not utilize the V9 gene segment. In addition, we have previously shown (5, 11, 17, 18, 21, 24, 25) that the majority of γδ TCR + T-cell lines derived from the peripheral blood of patients with primary immune deficiency disorders expressed primarily non-disulfide-linked γδ TCR. In contrast, most T-cell lines and 80 to 95% of T-cell clones derived from the peripheral blood of normal donors expressed mostly disulfide-linked γδ TCR utilizing the C1-1 gene segment (16–18). These results suggested a difference in the type of the γδ TCR expressed by T cells derived from the peripheral blood of patients with primary immune deficiency disorders and those derived from the peripheral blood of normal donors. This relatively increased expression of a non-disulfide-linked form of γδ TCR found in a number of other primary immunodeficiencies as well, especially severe combined immunodeficiency, is of great interest. It remains to be determined whether T cells expressing non-disulfide-linked γδ TCR have different properties than those expressing disulfide-linked γδ TCR and whether the former appear first in development.

The restricted TCR heterogeneity in Omenn’s syndrome is a common feature of both γδ and ωβ TCR (28). This observation suggests that although a restricted TCR heterogeneity is present, no predilection for expression of ωβ versus γδ TCR has been noted, suggesting a spectrum of abnormalities of lymphocytic lineage rather than a single defect. On the other hand, it has been suggested that this restricted heterogeneity of TCR expression actually represents a “leaky” variant of SCID, as has been described in SCID mice (3). This leakiness could result from extrathymic T-cell differentiation or escape of negative selection in the thymus (27) and subsequent specific antigen-driven selective expansion of “auto-immune” T-cell clones. Evidence can be cited both in favor and against either of these hypotheses, and it remains to be shown which is the prevailing mechanism.

Identification of the molecular and cellular defects in Omenn’s syndrome is critical for our understanding of the pathogenesis of the disease and of the molecular basis of the immunodeficiency as well as the steps of T-cell differentiation in vivo.

This work was supported in part by grant RO1 AR41003 from the National Institutes of Health.

REFERENCES


12. Ioannides, C. K., K. Itoh, F. E. Fox, R. Pahwa, R. A. Good, and C. D.


