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COMPARISON OF T CELL RECEPTOR GENE REARRANGEMENTS IN PATIENTS WITH LARGE GRANULAR T CELL LEUKEMIA AND FELTY'S SYNDROME[1]

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Felty's syndrome (FS) refers to the occurrence of rheumatoid arthritis, splenomegaly, and neutropenia. A subset of these patients has recently been described with a chronic T cell leukemia of large granular lymphocytes (LGCL). To examine the spectrum of lymphocyte abnormalities in FS and LGCL, we examined phenotypic and genotypic properties of lymphocytes from eight FS patients. In two of these FS patients, we observed an elevated proportion of T cells with an unusual phenotype ($CD3^+$/$Leu\text{-}7^+$/$Leu\text{-}8^-$/$CR\ 3^+$) (46 ± 5% of mononuclear cells). The FS lymphocytes had large granular morphology on Wright-Giemsa stain and were active in antibody-dependent cellular cytotoxic activity. This phenotype, morphology, and activity was similar to LGCL patients except that the latter T cells additionally expressed the Fc-IgG receptor recognized by monoclonal antibody Leu-11 (CD 15). In the remaining six FS patients, the proportion of $CD3^+$/$Leu\text{-}7^+$/$CR\ 3^+$ T cells was only 10 ± 8%, which was not significantly different from age-matched normal subjects (6.6 ± 2.2%). To determine the clonality of T lymphocytes in FS and LGCL, we examined DNA for rearrangements of the T cell antigen receptor β-chain (Ti_β) and γ-chain (Ti_γ) genes by using Southern blotting techniques. We found a clonal rearrangement of the $Ti_{\beta 1}$ and Ti_γ genes in both LGCL patients. In contrast, no clonal rearrangements of Ti_β or Ti_γ genes were detected in lymphocytes from the FS patients. These results indicate that FS patients are heterogenous in their phenotype and that one subset exhibits polyclonal expansion of an unusual lymphocyte subset.

Lymphoproliferation may occur at extra-articular sites in patients with rheumatoid arthritis (RA).[3] An example of such lymphoproliferation is the occurrence of splenomegaly and neutropenia in some RA patients, a combination that is termed Felty's syndrome (FS) (1, 2). In recent studies, a subset of these patients has been identified whose blood, bone marrow, and spleen contain a markedly increased proportion of T cells defined by a particular morphology (large granular lymphocytes with azurophilic granules) and cell surface markers defined by anti-CD8 (Leu-2/OKT8) monoclonal antibodies (MoAb) (3). The expansion of a particular lymphoctye subset in these patients raises the possibility that this disorder could derive in part from the expansion of an abnormal T cell clone. Alternatively, the large granular lymphocytes could represent a polyclonal proliferation of lymphocytes with homogeneous phenotype.

The clonality of T cell populations can be determined by analysis of genes encoding the T cell antigen receptor (Ti) (4–6). The Ti gene complex consists of α-, β-, and γ-chains that rearrange in mature T cells (7–12). Rearrangements of the Ti_β gene, and more recently the Ti_γ gene, have provided a marker for the clonality of acute and chronic T cell leukemias (12–15). These techniques were recently applied to lymphocytes from patients with an unusual syndrome called T cell lymphocytosis with neutropenia, where the clinical features did not allow a clear distinction between a chronic leukemia and a polyclonal proliferation of reactive T lymphocytes (16–19). The finding of a clonal rearrangement involving the Ti_β genes in at least some of these patients allowed recognition of this syndrome as a chronic leukemia called large granular T cell leukemia (LGCL) (13–15).

Some patients with FS are strikingly similar to LGCL with regard to a) the occurrence of lymphocytosis, splenomegaly, and profound neutropenia; and b) increased levels of large granular lymphocytes expressing the CD8 (Leu-2/OKT 8) and Leu-7 cell surface marker (20–22). Of importance, some of the LGCL patients have features of RA, suggesting that the leukemia has evolved in the setting of preexisting FS (17, 19, 20). In this study we have defined the phenotype of blood and splenic lymphocytes from two patients with LGCL and eight patients with FS. Two patients with LGCL and two with FS were shown to have increased levels of lymphocytes coexpressing the Leu-2 and Leu-7 cell surface markers. The clonality of lymphocytes from these patients was assessed by using Ti_β and Ti_γ gene rearrangements.

MATERIALS AND METHODS

Patients. FS is diagnosed when patients show classic RA, leukopenia, and splenomegaly. Eight patients with FS had granulocyto-

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[2] Correspondence to: Dr. Bruce Freimark, Department of Basic and Clinical Research, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Rd. - BCR4 La Jolla, CA 92037.
[3] Abbreviations used in this paper: FS, Felty's syndrome; LGCL, large granular T cell leukemia; Ti_β, β-chain of the T cell antigen receptor; PBL, peripheral blood lymphocyte; PE, phycoerythrin; C_β, constant region of the Ti_β; J_β, joining region of the Ti_β; D_β, diversity region of Ti_β; Ti_γ, γ-chain of the T cell antigen receptor; J_γ, joining region of the Ti_γ; MoAb, monoclonal antibody; E:T, effector to target cell; RA, rheumatoid arthritis.

penia (<2000/mm^3), splenomegaly, high (≥1:640) titer rheumatoid factor, and erosive changes on joint x-ray. Their granulocytopenia and splenomegaly could not be attributed to drug reactions, malignancies, hepatic cirrhosis, amyloidosis, or chronic infections, including tuberculosis. LGCL was diagnosed in two patients with chronic T cell lymphocytosis, neutropenia, and splenomegaly as described by Loughran et al. (20). The clinical features of two LGCL patients (cases 1 and 2) and two FS patients with an elevated proportion of large granular lymphocytes (cases 3 and 4) are described below.

Case 1. MAR is a 39-yr-old female with neutropenia and T cell lymphocytosis. In 1981 she developed Coombs-positive hemolytic anemia, which was refractory to glucocorticoid treatment but responded to splenectomy. Histologically, the spleen showed reactive lymphoid infiltrates, and no lymphoma was noted. She was in good health until June 1984, when she developed an infection and was noted to have a white blood cell count (WBC) of 17,500 containing >95% lymphocytes and <1% granulocytes. No lymph nodes or masses were present on physical examination or radiologic studies. Bone marrow aspirates and biopsies showed histologically normal early myeloid cells but absence of mature granulocytes. A lymphoid infiltrate was present in the bone marrow (approximately 30% of cells) that had phenotype similar to that in her blood (described below). A punch skin biopsy was used to establish a fibroblast culture line to serve as a source of germline DNA.

Case 2. ELA is a 71-yr-old male with adult onset diabetes mellitus who was admitted with fever and staphylococcal osteomyelitis of the left foot. Routine admission studies revealed a WBC of 5,800 with 75% lymphocytes 25% monocytes and <1% granulocytes. No enlarged lymph nodes were noted on routine physical or radiographic exams. Bone marrow aspirate was similar to that noted in case 1. After resolution of this infection, his chronic lymphocytosis and splenomegaly persisted. Several months later, he developed an episode of septicemia due to Gram-negative rods and expired.

Case 3. THO is a 61-yr-old white male with chronic RA involving feet, ankles, and proximal joints of his hands. He had positive rheumatoid factor (1:640), anti-nuclear antibody (1:640), and leukopenia (WBC 3800/mm^3 with <1% granulocytes). Bone marrow aspirate showed maturation arrest of myeloid precursors at the myelocyte stage. Leukopenia persisted despite treatment with nonsteroidal drugs and glucocorticoids. Because of persistent infections, the patient underwent splenectomy.

Case 4. MST is a 63-yr-old female with chronic RA with neutropenia (WBC 3,500 with <2% granulocytes). Additional extra-articular features included keratoconjunctivitis sicca (Sjogren's syndrome), vasculitis, peripheral neuropathy, and hypothyroidism. Serologic studies were positive for rheumatoid factor (1:10, 256), antinuclear antibody (1:320), and anti-thyroglobulin antibody. Because of recurrent infections, the patient underwent splenectomy.

Human cell lines and lymphocytes. Lymphocytes were prepared from peripheral blood, thymus, and spleen by Ficoll-Hypaque gradient centrifugation (23). Purified T cells were isolated by rosetting lymphocytes with neuraminidase-treated sheep red blood cells followed by separation on Ficoll-Hypaque gradients. After a second rosetting step, the red cells were lysed with Tris-buffered ammonium chloride (pH 7.5), washed with 0.9% saline and resuspended in RPMI 1640 with 10% fetal calf serum (FCS). Human tumor cell lines and primary fibroblast cultures were maintained in 75-cm^3 flasks (Costar 3075) with RPMI 1640 10% FCS and antibiotics.

Peripheral blood smears or cytocentrifuge preparations of purified lymphocytes from LGCL or FS patients were stained with Wright-Giemsa method. The presence of monocytes was determined with stains for nonspecific esterase (24).

MoAb and flow cytometry. MoAb included anti-Leu-2a (T suppressor/cytotoxic cells) (CD8), anti-Leu-3a (T helper/inducer cells) (CD4), anti-Leu-7 (subset of large granular lymphocytes), anti-Leu-11 (Fc receptor for IgG, CD16), anti-Leu-12 (mature B cells) (CD19), anti-Leu-15 (complement receptor CR3), anti-HLA-DR, and anti-Leu-M5 (monocytes) (Becton Dickinson, Mountain View, CA). For flow cytometry, 1×10^6 cells were incubated with the specific MoAb or with a control murine myeloma followed by fluorescein isothiocyanate- (FITC) labeled anti-mouse IgG (Tago, Burlingame, CA). For two-color fluorescent studies, FITC-conjugated anti-Leu-4, Leu-7, or HLA-DR plus phycoerythrin- (PE) conjugated anti-Leu-4, Leu-7, Leu-11a, or Leu-15 were used. Antibodies were deaggregated for 1 hr immediately before use in an airfuge (Beckman). Cells were analyzed by using a FACS IV flow cytometer and a consort 30 computer (Becton Dickinson).

In vitro functional analysis of lymphoid cells. Lymphoid cells from normal subjects, LGCL patients, or FS patients were evaluated for antibiotic dependent cellular cytotoxic (ADCC) and natural killer (NK) activity as described (25). Briefly, ADCC activity was measured by using ^{51}C-labeled lymphoblastic cell line JY and a murine IgG2a anti-HLA MoAb (ME-19, 5μg/ml) reactive with this cell line; all measurements were performed in triplicate at effector to target (E:T) ratios of 10:1 and 20:1, on the basis of prior studies performed with these target cells used at E:T ratios ranging from 0.4:1 to 100:1. NK assays were performed by using ^{51}Cr-labeled K562 cells at E:T ratios of 10:1 and 50:1, on the basis of prior studies performed by using E:T ratios of 2.5:1, 10:1, 50:1, and 100:1. The results in each case are expressed as proportion of total ^{51}Cr released after subtraction of spontaneous release during a 4-hr assay at 37°C.

Probes. The Ti_β cDNA clone YT35 (4), derived from the human T cell tumor line Molt-3, was generously provided by Dr. Tak Mak, Ontario Cancer Institute, Toronto, Ontario, Canada. A 700-bp fragment corresponding to the constant region of the Ti_β gene was isolated from YT35 by using the restriction endonucleases BglII and PSTI (Boehringer-Mannheim, Indianapolis, IN) and was subcloned into pUC-13. This probe hybridizes to both $C_{\beta 1}$ and $C_{\beta 2}$ of the β-chain gene. The J_γ probe is a 1-Kb subgenomic fragment subcloned into SP6, which encodes J_2 (12). This probe hybridizes to both J_1 and J_2 gene regions. Inserts were excised from plasmids and ^{32}P labeled by nick translation to specific activities of 1 to 3×10^8 cpm/μg DNA.

Southern blot analysis. Southern blot analysis of DNA from human cell lines and lymphocytes with the Ti_β and Ti_γ probes was performed as described (26). Genomic DNA was extracted from cells and was digested overnight with 10 U of restriction enzyme per microgram of DNA, was electrophoresed through 0.8% agarose gels, and was transferred to nitrocellulose. After transfer, filters were prehybridized overnight at 42°C in the presence of 50% formamide, 5× Denhardts solution, 100 μg/ml salmon sperm DNA, 3× SSC, 0.1% SDS, and 0.1% NaPPi and then were hybridized for 24 to 28 hr at 42°C with probe in prehybridization mixture at 3.0 to 5.0×10^6 cpm/ml. After two low-stringency washes at room temperature with 2× SSC/0.1% SDS, the blots were washed twice in 0.1× SSC/0.1% SDS for 30 min at 65°C. Blots were exposed for 24 to 96 hr to Kodak X-Omat AR film at −70°C, using Dupont Lighting Plus intensifying screens.

RESULTS

Phenotype of cell surface antigens. Cell surface antigens were determined by using MoAb and flow cytometry (Table I). Peripheral blood lymphocytes (PBL) from the two patients with LGCL (cases 1 and 2) had a significantly elevated proportion of Leu-2$^+$ (CD8), Leu-7$^+$, and Leu-11$^+$ (CD16) cells as compared with age-matched normals ($p < 0.05$). By two-color immunofluorescence (Fig. 1), these antigens were present on T cells as demonstrated by Leu-4$^+$ Leu-7$^+$ cells (*frame D*) and Leu-4$^+$ Leu-11$^+$ cells (*frame B*). Virtually all of the Leu-7$^+$ cells also expressed Leu-4$^+$ (CD3) (*frame D*). These results are in contrast to normal PBL (Table I), where a lower proportion of Leu-4$^+$ Leu-7$^+$ cells were present and where Leu-4$^+$ Leu-11$^+$ cells were not detected. PBL from two of the patients with FS (cases 3 and 4) also showed a markedly increased proportion (46% ± 5) of Leu-4$^+$ Leu-7$^+$ cells (Fig. 2, *frame C*), but Leu-4$^+$ Leu-11$^+$ cells (*frame B*) were not detectable. The intensity of MoAb Leu-7 staining in the PBL of cases 3 and 4 was less than for the LGCL patients (cases 1 and 2). In contrast, the other six patients with FS had only a slightly increased proportion of Leu-4$^+$ Leu-7$^+$ cells (10%

TABLE I

Phenotype of lymphocytes from patient's neutropenia and T cell lymphocytosis

MoAb	Cluster Designation	Case 1 PBL	Case 2 PBL	Case 3 Spleen	Case 4 PBL	Normal PBL
Leu-2	(CD8)	82	70	43	54	31 ± 4
Leu-3	(CD4)	7	4	21	10	50 ± 6
Leu-4	(CD3)	92	78	73	63	71 ± 5
Leu-12	(CD19)	8	11	24	27	7 ± 5
Leu-8		11	8	7	9	55 ± 5
Leu-11	(CD19)	29	40	2	<2	12 ± 3
Leu15	(CD11)	35	42	44	—	18 ± 1
Leu-4$^+$ Leu-11$^+$		27	39	<1	<1	<1
Leu-4$^+$ Leu-7$^+$		30	35	41	51	6.6 ± 2.2
Leu-4$^+$ Leu-15$^+$		31	—	44	55	8 ± 6

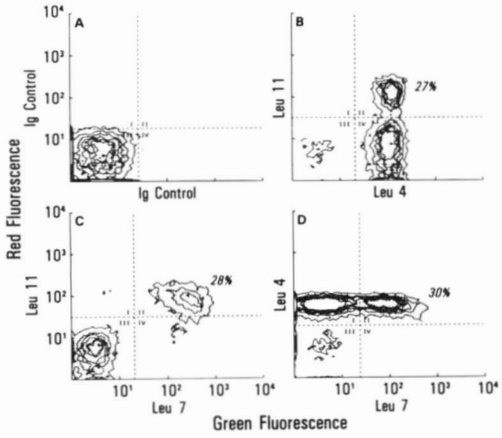

Figure 1. Two-color flow cytometry analysis of Leu-7, Leu-4, and Leu-11 expression on PBL derived from a LGCL patient. PBL from case 1 were stained with PE-Ig control and FITC-Ig control (*A*), PE-Leu-11a and FITC-Leu-4 (*B*), PE-Leu-11a and FITC-Leu-7 (*C*), and PE-Leu-4 and FITC-Leu-7 (*D*). Data are shown as log scale of PE and FITC fluorescence. The percentage of PBL that stain with both fluorochromes is indicated in each panel.

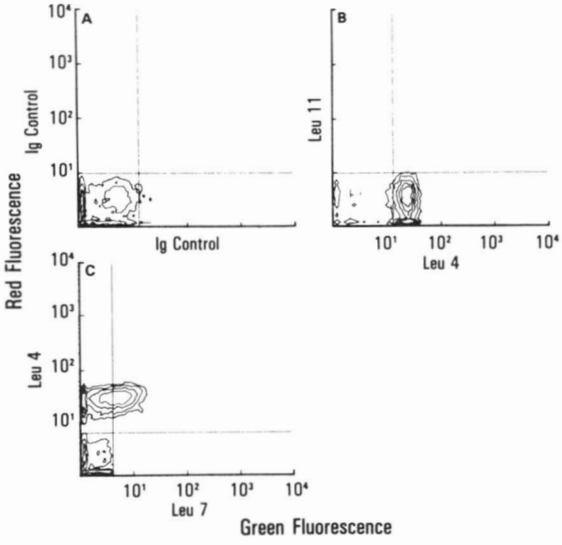

Figure 2. Two-color flow cytometry analysis of Leu-4, Leu-7, and Leu-11 expression on spleen cells derived from a patient with FS. Spleen lymphocytes were stained with PE-Ig control and FITC-Ig control (*A*, PE-Leu-11 and FITC-Leu-4 (*B*) and PE-Leu-4 and FITC-Leu-7 (*C*). Data are shown as log peak of PE and FITC fluorescence.

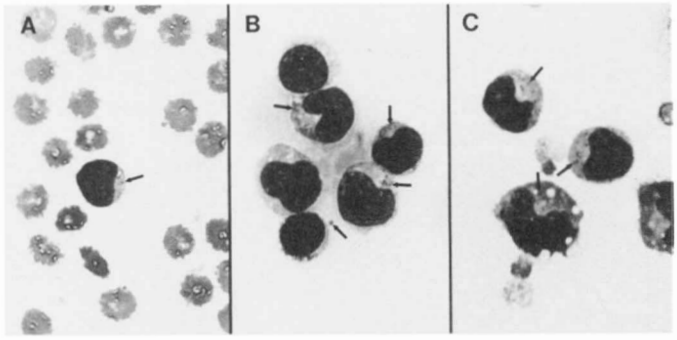

Figure 3. Peripheral blood from a LGCL patient (*A*) and cytocentrifuge preparations of lymphocytes from two FS patients (*B* and *C*, respectively) were air-dried and stained by the Wright-Giemsa method (24). Arrows indicate granules in the cytoplasm. Original magnification 400×.

± 8) that was not significantly ($p < 0.01$) greater than found in age-matched controls.

These results demonstrated an increased proportion of Leu-4$^+$ Leu-7$^+$ cells in cases 1 through 4. Because each

TABLE II

Cytotoxic activity of lymphocytes

Source	ADCC		NK Activity	
	E:T 10:1	E:T 20:1	E:T 10:1	E:T 50:1
Normal[a]	21 ± 5	35 ± 6	15 ± 4	52 ± 11
LGCL	55 ± 8	89 ± 6	3 ± 1	4 ± 1
FS-Case 1	49 ± 9	85 ± 10	10 ± 3	32 ± 4
FS-Case 2	40 ± 13	81 ± 13	9 ± 3	26 ± 6

[a] Lymphocytes were obtained from blood of normal subjects or from patients with LGCL or FS.

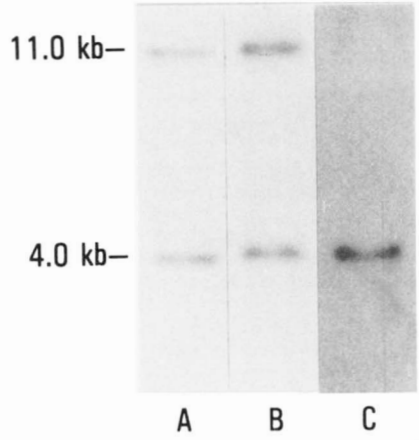

Figure 4. Analysis of Ti_β gene rearrangements in germline and polyclonal T cell DNA. High molecular DNA prepared from normal salivary gland (*A*), fibroblasts from patient MAR (*B*), and normal thymus (*C*) was digested with Eco RI and analyzed for Ti_β gene rearrangement by using Southern blot analysis as described in *Materials and Methods*.

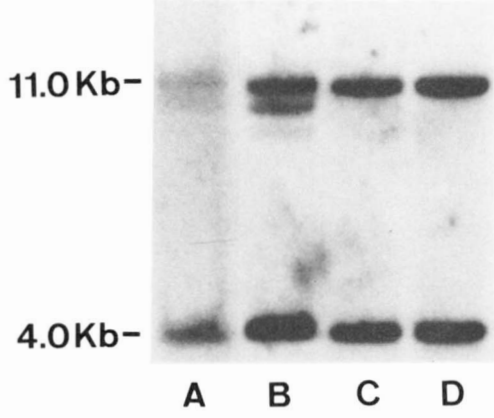

Figure 5. Analysis of patient lymphocyte DNA for $Ti_{\beta 1}$ gene rearrangements. Lymphocyte DNA prepared from patient ELA (*A*), MAR (*B*), THO (*C*), and MST (*D*) was digested with Eco RI and subjected to Southern blot analysis performed with a cDNA specific for the constant region of the Ti_β gene. The markers indicate the position of DNA in the germline configuration.

of these patients had lymphocytosis (lymphocytes >3000/mm^3), they had an increased number of cells with this phenotype. Additional cell surface markers that subdivide the Leu-2$^+$ (CD8$^+$) population of T cells include the Leu-15 (CR3) and Leu-8 antigens (27). These MoAb were used to further characterize the phenotype in cases 1 to 4 as Leu-2$^+$/Leu-4$^+$/Leu-7$^+$/Leu-8$^-$/Leu-15$^+$ (Table I). Of importance, the proportion of cells with this phenotype in normal PBL was only 6.6 ± 2.2. Further, the proportion of cells with this phenotype in normal spleen was 5.0 ± 2.0.

Lymphocytes from LGCL patients contain small cytoplasmic granules (see Fig. 3, *frame A*) on Wright-Giemsa stain. Cytoplasmic granules were also seen in cytocentri-

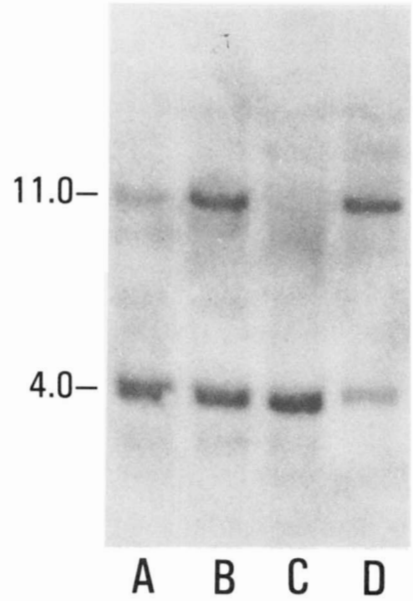

Figure 6. Analysis of $Ti_{\beta 1}$ rearrangements in lymphocyte DNA obtained from FS patients. Lymphocytes were separated into T cell and non-T cell fractions before DNA extraction, digested with Eco RI, and analyzed by Southern blot analysis performed with the C_β probe. *A*, Patient THO splenic T cells; *B*, patient THO splenic non-T cells; *C*, patient MST PBL T cells; *D*, patient MST PBL non-T cells.

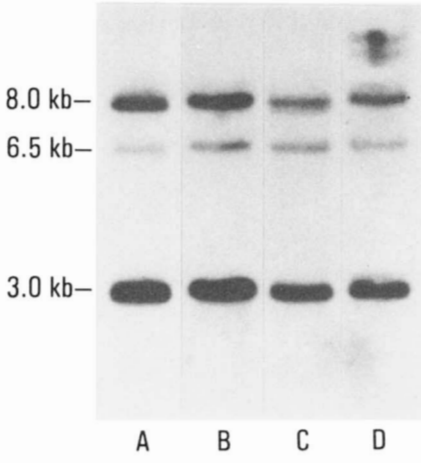

Figure 7. Analysis of patient lymphocyte DNA for $Ti_{\beta 2}$ gene rearrangements. DNA prepared from granulocytes (*A*), patient THO lymphocytes (*B*), patient MST lymphocytes (*C*), and patient MAR PBL (*D*) was digested with Hind III and analyzed for $Ti_{\beta 2}$ gene rearrangements by using Southern blot analysis and Ti_β constant region-specific cDNA probe. Markers indicate the position of DNA in the germline configuration.

fuge preparations of lymphoid cells from the FS patients (*frames B and C*). Although these cells had somewhat monocytoid appearances, they were negative on staining for nonspecific esterase (unpublished observations) and were reactive with MoAb directed against T cells as described above.

In vitro functional properties. LGCL expressed ADCC activity (Table II) but little NK activity, in agreement with results of previous studies (21). The lymphoid cells from the FS patients also were able to effect ADCC activity. They also exhibited some NK activity, although significantly ($p < 0.05$) less than age-matched normal PBL.

Rearrangements of the Ti_β genes. To ascertain the clonality of lymphocytes from these patients, we first analyzed DNA for Ti_β gene rearrangements by using a C_β gene probe. Germline DNA from normal salivary gland (Fig. 4, *lane A*) digested with EcoRI contains an 11.0-Kb

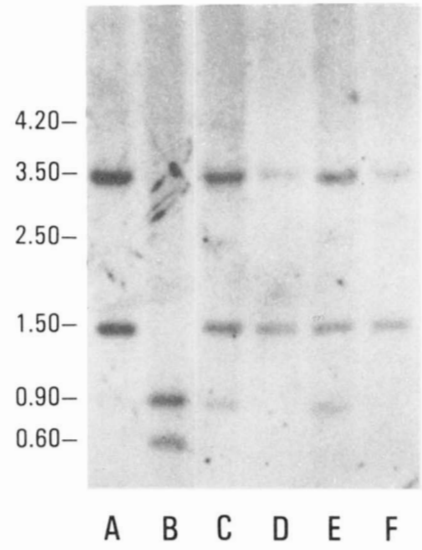

Figure 8. Analysis of Ti_γ gene rearrangements in lymphocyte DNA obtained from FS patients. Lymphocyte DNA was prepared as described in the legend of Figure 5 and digested with Eco RI. Southern blot analysis was performed with a J_γ probe. *A*, Granulocytes (germline); *B*, RPMI-8402; *C*, patient THO splenic T cells; *D*, patient THO splenic non-T cells; *E*, patient MST PBL T cells; *F*, patient MST PBL non-T cells.

fragment that includes the $C_{\beta 1}$ and $J_{\beta 1}$ genes and a 4.0-Kb band containing $C_{\beta 2}$. Fibroblast DNA from patient MAR (case 1 with LGCL, *lane B*) contained bands in this germline configuration; similar bands were also noted in granulocytes from patient ELA (case 2 with LGCL) (data not shown). In comparison, normal thymocytes completely rearranged their $C_{\beta 1}$ genes, as evidenced by decreased intensity of the 11.0-Kb band (*lane C*).

DNA extracted from the LGCL patients (*lanes A and B*, respectively, in Fig. 5) both exhibited an additional band at approximately 10.0 Kb. In contrast, DNA extracted from FS lymphocytes (*lanes C and D*) did not show any detectable rearranged band. The high proportion of 11-Kb bands might result from non-T cells in the lymphocyte preparation from unrearranged $C_{\beta 1}$ allele, or from a rearranged $C_{\beta 1}$ allele with size 11 Kb. To help resolve these possibilities, lymphocytes from FS patients were separated by rosetting methods into T cell (>90% Leu-4^+ by flow cytometry) and non-T cell fractions. Purified T cell DNA from FS patient THO (Fig. 6, *lane A*) exhibited a slightly diminished 11.0-Kb band in comparison to the 11.0-Kb band of non-T cell DNA (*lane B*). In contrast, T cell DNA from patient MST (*lane C*) demonstrated nearly complete loss of the 11.0-Kb band in comparison with the 11.0-Kb band of non-T cell DNA (*lane D*).

Possible rearrangements involving the $C_{\beta 2}$ and $J_{\beta 2}$ alleles were investigated by using Hind 3 digestion. Germline configuration of normal granulocyte DNA includes 3-, 6.5-, and 8-Kb bands (*lane A*, Fig. 6). The 3-Kb band corresponds to the $C_{\beta 1}$ gene, the 6.5-Kb band consists of the 3′ end of the $C_{\beta 2}$, and the 8-Kb fragments contain the $C_{\beta 2}$ and $J_{\beta 2}$ segments. DNA from the LGCL patient shows the appearance of additional bands at 10 Kb and 11 Kb (lane D in Fig. 7). DNA samples from the patients with FS (*lanes B and C*) were similar to the germline configuration.

Rearrangement of the Ti_γ gene. To determine if T cells from FS patients express Ti gene rearrangements not detected by using the C_β probe, blotted DNA was digested with EcoRI and reprobed with a J_γ gene probe. The J_γ

probe hybridizes to germline $J_{\gamma 1}$ and $J_{\gamma 2}$ regions, 1.5 and 3.5 Kb, respectively (Fig. 8, *lane A*). Clonal T cell DNA (RPMI-8402, *lane B*) expresses two distinct rearranged bands and loss of both germline bands. T cell DNA obtained from both FS patients (*lanes C and E*) expressed both germline J region bands, a rearranged band 0.9 Kb, and faint bands at 2.5 and 4.2 Kb (seen on longer exposure of autoradiography). The non-T cells from the FS patients (*lanes D and F*) showed germline configuration. The reactivity of J_γ probe with FS T cell and non-T cell DNA was similar to that seen with DNA samples from normal T cells and non-T cells, respectively (unpublished observations).

DISCUSSION

In recent studies, an association between LGCL and FS has been noted (19, 20). This suggested the possibility that FS could arise as a consequence of expansion of an abnormal clone of T cells that mediate granulocyte destruction. In agreement with results of other studies (13, 14), we found evidence of clonality of T cells in two patients with LGCL by using probes for the Ti_β and Ti_γ genes. Among eight patients with FS, we found two patients whose lymphocytes exhibited phenotypic similarity to LGCL patients. Lymphoid cells from these FS patients exhibited morphologic and functional similarities to the LGCL patients. However, T cell DNA from these FS patients did not exhibit clonal rearrangements of their T cell antigen receptor genes. Therefore, we conclude that the increased proportion of large granular lymphocytes in these FS patients results from a polyclonal expansion of lymphocytes with a similar phenotype.

T cell DNA from one FS patient showed a suprisingly large proportion of T_β gene with size 11 Kb after Eco RI digestion. This observation may have several explanations. First, these T cells may have been contaminated with non-T cells (that contain an 11-Kb germline $C_{\beta 1}$ gene) before DNA extraction. This seems unlikely, since the proportion of T cells determined by flow cytometry was greater than 90%. Second, the 11-Kb band may be a rearranged $C_{\beta 1}$ allele with a size similar to that of germline. However, this seems unlikely, since no evidence for clonal rearrangement was noted when the FS T cell DNA was analyzed for rearrangements of the Ti_γ genes. Finally, the 11-Kb $C_{\beta 1}$ band may result from one unrearranged $C_{\beta 1}$ allele in the T cells. Schmidt et al. (28) have shown that some T cell clones with NK activity have one $C_{\beta 1}$ allele in the germline configuration. Thus, the increased 11-Kb band in FS T cells may reflect the persistence of one non-rearranged allele.

The mechanisms responsible for neutropenia in LGCL and in FS have remained controversial. Some studies have reported anti-granulocyte antibodies and an ADCC mechanism (19). In support of this mechanism, the LGCL cells possess a receptor for the Fc portion of IgG (recognized by MoAb Leu-11) and exhibit ADCC when tested in vitro. However, the T cells in our FS patients lacked the Fc receptor defined by MoAb Leu-11 (CD 16). This suggests that such cells may possess another type of Fc receptor, the receptor is blocked from detection in vivo, or that our observed ADCC activity during in vitro assays resulted from another subpopulation of mononuclear cells. In addition to ADCC as a mechanism for explaining granulocytopenia in FS, other investigators (10) have proposed destruction of granulocytes by the direct cytotoxic action of the large granular lymphocytes. It is likely that both mechanisms are operative in most patients, and further characterization of T cell receptor on cloned T cells from these patients will allow characterization of the relative contribution of each mechanism.

In summary, expansion of an unusual subset of lymphocytes (Leu-4^+/Leu-7^+) occurs in patients with lymphocytosis, splenomegaly, and neutropenia. These patients include a spectrum that ranges from polyclonal proliferation of this subset (in most FS patients) to frank neoplastic proliferation of a clonally restricted T cell (in LGCL patients). These clinical conditions provide an unusual opportunity to examine the T cell events that occur in patients with autoimmune disease and their neoplastic counterparts.

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REFERENCES

1. **Goldberg, J., and R. S. Pinals.** 1980. Felty syndrome. *Semin. Arthritis Rheum. 10:52.*
2. **Barnes, C. G., A. L. Turnbull, and B. Vernon-Roberts.** 1971. Felty's syndrome. A clinical and pathological survey of 21 patients and their response to treatment. *Ann. Rheum. Dis. 30:359.*
3. **Linch, D., A. Newland, A. Turnbull, L. Knott, A. Machannel, and P. Beverly.** 1984. Unusual T cell proliferations and neutropenia in rheumatoid arthritis: comparison with classical Felty's syndrome. *Scand. J. Haematol. 33:342.*
4. **Toyonaga, B., Y. Yanagi, N. Suciu-Foca, M. Minden, and T. W. Mak.** 1984. Rearrangements of T cell receptor gene YT35 in human DNA from thymic leukaemia T cell lines and functional T cell clones. *Nature 311:385.*
5. **Minden, M. P., B. Toyonaga, K. Ha, Y. Yanagi, B. Chin, E. Gelfand, and T. Mak.** 1985. Somatic rearrangement of T cell antigen receptor gene in human T cell malignancies. *Proc. Natl. Acad. Sci. USA 82:1224.*
6. **Flug, F., P. G. Pelicci, F. Bonetti, D. M. Knowles, and R. Dalla-Favera.** 1985. T cell receptor gene rearrangements as markers of lineage and clonality in T cell neoplasms. *Proc. Natl. Acad. Sci. USA 82:3460.*
7. **Kronenberg, M., G. Siu, L. E. Hood, and N. Shastri.** 1986. The molecular genetics of the T cell antigen receptor and T cell antigen recognition. *Annu. Rev. Immunol. 4:529.*
8. **Yoshikoi, Y., S. Clark, S. Taylor, V. Sohn, B. Wilson, M. Minden, and T. Mak.** 1985. Organization and sequences of the variable, joining and constant region genes of the human T cell receptor alpha chain. *Nature 316:837.*
9. **Tunnacliffe, A., R. Kefford, C. Milstein, A. Forster, and T. H. Rabbitts.** 1985. Sequence and evolution of the human T cell antigen receptor beta-chain genes. *Proc. Natl. Acad. Sci. USA 82:5068.*
10. **Royer, H. D., O. Acuto, M. Fabbi, R. Tizard, K. Ramachandran, J. E. Smart, and E. L. Reinherz.** 1984. Genes encoding the Ti-beta subunit of the antigen/MHC receptor undergo rearrangement during intrathymic ontogeny prior to surface T3-Ti expression. *Cell 39:261.*
11. **Kranz, D. M., H. Saito, M. Heller, Y. Takagaki, W. Haas, H. N. Eisen, and S. Tonegawa.** 1985. Limited diversity of the rearranged T cell gamma gene. *Nature 313:752.*
12. **Quertermous, T., C. Murre, D. Dialynas, A. Duby, J. Strominger, T. Waldman, and J. Seidman.** 1986. Human T cell γ-chain genes: organization, diversity and rearrangement. *Science 231:252.*
13. **Aisenberg, A., T. Krontiris, T. Mak, and B. Wilkes.** 1985. Rearrangement of the gene for the beta-chain of the T cell receptor in T cell chronic lymphocytic leukemia and related disorders. *N. Engl. J. Med. 313:529.*
14. **Waldmann, T., M. Davis, K. Bongiovanni, and S. Korsmeyer.** 1985. Rearrangements of genes for the antigen receptor on T cells as

markers of lineage and clonality in human lymphoid neoplasms. *N. Engl. J. Med. 313:776.*

15. **Berliner, N., A. Duby, D. Linch, C. Murre, T. Quertermous, L. Knott, T. Azin, A. Newland, D. Lewis, M. Galvin, and J. Seidman.** 1986. T cell receptor gene rearrangements define a monoclonal T cell proliferation in patients with T cell lymphocytosis and cytopenia. *Blood 67:914.*
16. **Gale, R. P., and K. A. Foon.** 1985. Chronic lymphocytic leukemia. *Ann. Intern. Med. 103:101.*
17. **Newland, A. C., D. Catovsky, D. Linch, J. C. Cawley, P. Beverley, J. F. San Miguel, E. C. Gordon-Smith, T. E. Blecher, S. Shahriari, and S. Varadi.** 1984. Chronic T cell lymphocytosis: a review of 21 cases. *Br. J. Haematol. 58:433.*
18. **Pandolfi, F., G. Semenzato, G. DeRossi, D. M. Strong, I. Quinti, A. Pezzutto, F. Mandelli, and F. Auiti.** 1982. Heterogeneity of T-CLL defined by monoclonal antibodies in nine patients. *Clin. Immunol. Immunopathol. 24:330.*
19. **Starkebaum, G., P. J. Martin, J. W. Singer, L. G. Lum, T. H. Price, M. E. Kadin, W. H. Raskind, and P. J. Fialkow.** 1983. Chronic lymphocytosis with neutropenia: evidence for a novel, abnormal T cell population associated with antibody-mediated neutrophil destruction. *Clin. Immunol. Immunopathol. 27:110.*
20. **Loughran, T. P., M. E. Kadin, G. Starkebaum, J. Abkowitz, E. Clark, C. Disteche, L. Lum, and S. Slichter.** 1985. Leukemia of large granular lymphocytes: association with clonal chromosomal abnormalities, and autoimmune neutropenia, thrombocytopenia and hemolytic anemia. *Ann. Intern. Med. 102:169.*
21. **Schlimok, G., E. Thiel, E. P. Rieber, D. Huhn, H. Feucht, J. Lohmeyer, and G. Riethmuller.** 1982. Chronic leukemia with a hybrid surface phenotype (T lymphocytic/myelomonocytic): leukemic cells displaying natural killer activity and antibody-dependent cellular cytotoxicity. *Blood 59:1157.*
22. **Aisenberg, A. C., B. M. Wilkes, N. L. Harris, K. A. Ault, and R. U. Carey.** 1981. Chronic T cell lymphocytosis with neutropenia: report of a case studied with monoclonal antibody. *Blood 58:818.*
23. **Böyum, A.** 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest. 21(Suppl. 97):1.*
24. **Yam, L. J., L. Y. Li, and D. T. Yam.** 1982. Esterases in human leukocytes. *J. Histochem. Cytochem. 21:1.*
25. **Lanier, L., T. Kipps, and J. Phillips.** 1985. Functional properties of a unique subset of cytotoxic $CD3^+$ T-lymphocytes that express Fc receptors for IgG (Leu-11 antigen). *J. Exp. Med. 162:2089.*
26. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol. 98:503.*
27. **Lanier, L. L., and J. H. Phillips.** 1985. A map of the cell surface antigens expressed on resting and activated human natural killer cells. In *Leukocyte Typing.* T. E. Reinberg et al., eds. Springer-Verlag, New York. P. 157.
28. **Schmidt, R., G. Bartley, S. Lee, J. Daley, H. Royer, H. Levine, E. Reinherz, S. Schlossman, and J. Ritz.** 1986. Expressioin of the NKTa clonotype in a series of human natural killer clones with identical cytotoxic specificity. *J. Exp. Med. 163:812.*