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Research Article

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A Novel Lymphoproliferative/Autoimmune Syndrome Resembling Murine *lpr/gld* Disease

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Abstract

In mice, the two distinct autosomal recessive genes *lpr* and *gld* can induce a syndrome characterized by autoantibody formation and the progressive accumulation of an unusual $CD4^-CD8^-$ T cell population in peripheral lymphoid tissue. This phenotype does not precisely mirror any human disease. In this report we describe two patients with a progressive lymphoproliferative disorder associated with autoimmunity. The peripheral blood and lymph nodes of these patients contained large numbers of an unusual $CD4^-CD8^-$ T cell population. These $CD4^-CD8^-$ T cells express surface markers characteristic of mature peripheral blood T cells (CD3, CD2, CD5), express the α/β form of the T cell receptor, and do not express surface markers characteristic of immature thymocytes (CD1) or NK cells (CD16, CD56). Functionally, these cells exhibited deficient proliferation and lymphokine production upon stimulation with mitogenic antibodies to CD3 or CD2. Both proliferation and lymphokine production could be augmented by co-stimulation with an antibody directed at the CD28 determinant. The clinical and immunological features of this syndrome resemble the lymphoproliferative/autoimmune disease seen in *lpr* and *gld* mice. (*J. Clin. Invest.* 1992. 90:334–341.) Key words: autoimmunity • double-negative • human • T cell receptor α/β chains

Introduction

The vast majority of post-thymic mature T cells express the T cell receptor α and β chains (TCRα/β)[1] in association with the CD3 molecular complex. In addition to the TCRα/β, these mature T cells express either CD4 or CD8 surface glycoproteins which serve as accessory molecules involved in major histocompatibility complex class II and class I recognition, respectively. A small subpopulation of mature T cells express neither CD4 nor CD8. The majority of these "double negative" cells express an alternate form of the T cell receptor consisting of γ and δ chains (1). Recently, a very rare subpopulation of T cells that express the TCRα/β but do not express CD4 or CD8 (TCRα/β $CD4^-CD8^-$) have been identified in the thymus and peripheral lymphoid tissue of mice and humans (2–5). The biological significance of this unusual T cell subpopulation is unknown. Furthermore, the scarcity of these cells in peripheral lymphoid tissue has made it difficult to study their functional properties.

In mice, two distinct autosomal recessive genes, *lpr* and *gld*, can induce a lymphoproliferative syndrome characterized by the massive accumulation of nonmalignant TCRα/β $CD4^-CD8^-$ T cells in association with autoimmune disease (reviewed in reference 6). Because some of the autoimmune features of *lpr* and *gld* mice resemble human systemic lupus erythematosus, they have been used as models of this disease. However, the massive accumulation of TCRα/β $CD4^-CD8^-$ T cells in the liver, spleen, and lymph nodes of *lpr* and *gld* mice, is not a feature of human systemic lupus erythematosus.

In this report, we describe two children with a novel syndrome characterized by hypergammaglobulinemia, lymphocytosis, hepatosplenomegaly, prominent lymphadenopathy, and autoimmunity. The lymphocytosis and lymphadenopathy in these two patients was associated with a profound expansion of TCRα/β $CD4^-CD8^-$ T cells. The clinical and immunological features of these cases resemble *lpr* and *gld* disease in mice.

Case reports

Patient 1. This female child was the product of a 38-wk pregnancy occurring while the mother was on clomiphene citrate therapy for infertility. In the 6th mo, the mother was treated with bed rest and ritodrine for premature labor, but the pregnancy was otherwise uneventful. The patient's early development was normal and routine vaccinations for diphtheria, pertussis, tetanus, measles, mumps, rubella, and oral polio were given without incident.

At age 18 mo the patient developed cervical adenopathy. Serological studies for Epstein-Barr virus (EBV) and cytomegalovirus (CMV) were negative and no infectious etiology for the adenopathy could be found. A cervical lymph node biopsy showed only reactive hyperplasia. At age 24 mo she developed anemia associated with hepatosplenomegaly and peripheral lymphocytosis. A bone marrow examination showed erythroid hyperplasia. Over the next months hematuria, proteinuria, and renal insufficiency developed. A renal biopsy showed mesangiopathic glomerulonephritis with crescent formation. Because of persistent anemia felt to be secondary to hypersplenism, the patient underwent a splenectomy. A wedge biopsy of the liver performed concurrently showed focal lymphocytic infiltrates in the portal triads. After splenectomy the hemoglobin level stabilized and the renal insufficiency resolved, however, the lymphocytosis and lymphadenopathy persisted. Serological studies at this time for EBV, CMV, toxoplasmosis, brucella, human immunodeficiency virus type 1 (HIV-1) and hepatitis B were all negative.

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1. *Abbreviations used in this paper:* CMV, cytomegalovirus; TCR, T cell receptor; similarly, TCRα/β, α and β chains of the TCR.

The family history was remarkable only for a healthy sibling (aged 8 yr), and no family history of lymphoproliferative or autoimmune disorders.

In August 1990 the patient (then aged 3 yr) was referred to the National Institutes of Health for evaluation. Her weight was 12 kg and height 90 cm (both below the fifth percentile). Physical examination disclosed diffuse lymphadenopathy with multiple lymph nodes (each 2–4 cm in diameter) in the anterior and posterior cervical regions and slightly smaller lymph nodes in the preauricular, occipital, epitrochlear, and supraclavicular areas. Prominent lymph nodes were also found in both axillae and inguinal areas. The liver extended three fingerbreadths below the right costal margin. Computerized tomography of the chest and abdomen showed a normal amount of thymic tissue, hepatomegaly, and prominent mediastinal and retroperitoneal adenopathy. The white blood count was 28,900 with 15% neutrophils, 2% band forms, 54% lymphocytes, 6% atypical lymphocytes, 13% monocytes, and 10% eosinophils. Urinalysis showed trace protein and microscopic hematuria. Serologic studies were negative for antibodies to CMV, HIV, HTLV-1, EBV capsid antigen, early antigens, and nuclear antigen. IgM antibodies to parvovirus were negative; IgG antibodies were positive. Cryoglobulins and rheumatoid factor were undetectable but anti-nuclear antibodies were present at a titer of 1:320 in a nucleolar pattern.

Patient 2. This female patient was the product of an uncomplicated pregnancy. At age 9 mo anemia, reticulocytosis, and splenomegaly were noted. Hemolytic anemia was subsequently diagnosed on the basis of a bone marrow examination showing erythroid hyperplasia and positive direct Coomb's test. Over the next several years the patient was treated with varying doses of corticosteroids and numerous blood transfusions. At age 3 yr the patient underwent a splenectomy for treatment of the hemolytic anemia. At age 4 yr the patient developed peripheral lymphadenopathy. A lymph node biopsy showed reactive follicular hyperplasia and cultures for bacteria, mycobacteria, and fungi were negative. Serological testing for HIV antibodies was negative. Over the next 4 yr the lymphadenopathy persisted and the patient continued to exhibit significant hemolysis requiring low dose corticosteroid therapy.

In August 1991 the patient (then aged 8 yr) was referred to the National Institutes of Health for evaluation. Her weight was 14.6 kg and height was 125 cm. Physical examination disclosed hepatomegaly and diffuse lymphadenopathy similar to that seen in patient 1. Computed tomography of the chest and abdomen showed hepatomegaly, along with prominent mediastinal, mesenteric, and retroperitoneal adenopathy. The hemoglobin was 7.6 g/dl with a reticulocyte count of 3.5%. The white blood count was 15,700 with 29% neutrophils, 55% lymphocytes, and 10% monocytes. Cryoglobulins, rheumatoid factor, and anti-nuclear antibodies were undetectable. Direct Coomb's test was positive for C3 and IgG. Serological studies for EBV were positive with a IgG viral capsid antigen of 1:2560, IgM VCA < 1:8, and Epstein-Barr nuclear antigen (EBNA) of 1:80.

Methods

Peripheral blood mononuclear cells were separated from freshly drawn heparinized venous blood or buffy coat fractions by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation. T cells were isolated by twice rosetting peripheral blood mononuclear cells with neuraminidase treated sheep red blood cells. $CD4^-CD8^-$ T cells were purified from the patient's peripheral blood by a negative selection procedure. Initially, the T cell fraction was treated with the following murine monoclonal antibodies. OKT4 and OKT8 which recognize the CD4 and CD8 antigens, respectively; 3C10 (7) and 63D3 (8) which react with human monocytes; and THB5 (9) an anti-B cell antibody which recognizes CD21 (all hybridomas obtained from American Type Culture Collection, Rockville, MD). The cells were then incubated with magnetic beads coated with goat anti-mouse IgG (DYNAL, Inc., Great Neck, NY). Finally, the bead-negative population ($CD4^-CD8^-$ T cells) was recovered by magnetic separation as described (10).

Isolated peripheral blood mononuclear cell or purified $CD4^-CD8^-$ cells were stained with the fluorescein isothiocyanate-conjugated or phycoerythrin-conjugated murine monoclonal antibodies listed below, and analyzed by flow cytometry using standard techniques (11). Portions of the left cervical and right inguinal lymph nodes were submitted for routine histopathology, immunohistochemistry on both paraffin and frozen sections, and immunophenotypic analyses on cells in suspension by flow cytometry. The antibodies employed for immunofluorescent and immunoperoxidase staining (for peripheral blood and lymph node) were those binding to: Leu 4 (CD3), Leu 5 (CD2), Leu 1, (CD5), Leu 6 (CD1), Leu 2 (CD8), Leu 3ab (CD4), Leu 16 (CD20), Leu 11 (CD16), Leu 7 (CD57), Leu 19 (CD56), the IL-2 receptor (CD25), Leu 22 (CD43), and WT-31 (TCRα/β) (all obtained from Becton Dickinson & Co., Mountain View, CA); TCRδ-1 (TCRγ/δ) (T Cell Sciences, Cambridge, MA); 2H4 (CD45RA), T11 (CD2), B1 (CD20), and NKH-1 (CD56) (Coulter Immunology, Hialeah, FL); Goat anti-mouse IgG (Kirkegaard & Perry, Gaithersburg, MD); Ber H2 (CD30), L-26 (CD20), UCHL-1 (CD45RO), and Ki-67 (Dako Corp., Carpinteria, CA); and immunoglobulin heavy (IgM, IgD, IgG, and IgA) and light (κ and λ) chains (Bethesda Research Laboratories, Bethesda, MD). Portions of the lymph nodes were embedded in OCT (Miles Laboratories, Naperville, IL) and snap-frozen in a 2-methylbutane/dry ice bath. Air-dried, acetone-fixed frozen sections were stained by the avidin-biotin-complex method as previously described (12), as were deparaffinized B5 fixed sections. Lymph node tissue from both patients was examined for the expression of the EBV-associated antigens, EBNA-2 and LMP-1 as previously described (13).

Proliferative responses to antibodies and mitogens were assayed by standard techniques (11). Phorbol 12-myristate 13-acetate (PMA) was obtained from Sigma Chemical Co., St. Louis, MO. PHA was obtained from Gibco Laboratories, Grand Island, NY, and used at a final concentration of 1%. For anti-CD3-induced proliferation, monoclonal antibody 64.1 immobilized on plastic tissue culture plates was used as previously described (14). For anti-CD2-induced proliferation, antibodies $T_{11}2$ and $T_{11}3$ were used as previously described (15). For anti-CD28-induced proliferation, the monoclonal antibody 9.3 (16) was prepared as described (17), and used at a final concentration of 0.5 μg/ml. Recombinant IL-2 was obtained from Amgen, Inc., Thousand Oaks, CA.

To quantitate IL-2 and interferon-γ production in vitro, cells were cultured for 72 h as previously described (11). Supernatants were assayed for IL-2 by ELISA (New England Nuclear Research Products, Boston, MA), and for interferon-γ by ELISA (Amgen, Inc.) using the protocols supplied by the manufacturers.

TCR and immunoglobulin gene rearrangement analyses were performed on high molecular weight DNA extracted from peripheral blood mononuclear cells, digested with either EcoRI or HindIII, and analyzed by Southern blotting. The TCRβ probe employed recognizes both human β constant genes (18). To detect possible rearrangements of the immunoglobulin heavy chain genes, blots were probed with a 6-kb BamHI/HindIII genomic DNA fragment which spans the immunoglobulin J_H region (19). Both probes were labeled with ^{32}P by random priming.

Results

Analysis of lymphocyte surface markers. The surface phenotype of the two patient's peripheral blood lymphocytes is sum-

marized in Table I. Both patients exhibited a striking lymphocytosis owing to an increase in the absolute number of B lymphocytes and an increase in the absolute number of $CD3^+$ T cells expressing neither CD4 or CD8 ($CD4^-CD8^-$ T cells). On multiple determinations, between 40% and 60% of these patient's peripheral blood T cells were $CD4^-CD8^-$.

To determine whether a clonal expansion of T or B lymphocytes was present, DNA was extracted from peripheral blood lymphocytes from both patients, digested with restriction enzymes, and analyzed by Southern blotting. These blots were then hybridized with probes specific for either the immunoglobulin J_H region or the β chain of the T cell receptor. No clonally rearranged bands were detected (data not shown) indicating that the lymphocytosis in these patients was not due to a clonal expansion of B cells or of T cells expressing the α/β antigen receptor. In patient 1, this unusual pattern of lymphocytosis has been a consistent finding during 12 mo of follow-up.

The abundance of the $CD4^-CD8^-$ T cells in the peripheral blood of these patients allowed us to purify them by negative selection and examine their surface phenotype and functional properties. The $CD4^-CD8^-$ cells from both patients co-expressed surface markers characteristic of mature T cells including CD3 and CD5. They did not express CD25 (α chain of the IL-2 receptor) and, in the case of patient 1, did not express the thymocyte marker CD1, nor the NK cell markers CD16 and CD56 (patient 2 was not tested). To determine the nature of the TCR used by the double-negative cells, purified $CD4^-CD8^-$ cells from both patients were examined with monoclonal antibodies which recognize conserved determinants on either α/β of γ/δ TCR molecules. The results of such an experiment on purified $CD4^-CD8^-$ from patient 1 are shown in Fig. 1. Virtually identical histograms were obtained when $CD4^-CD8^-$ cells from patient 2 were stained with the same antibodies (data not shown and Table II). As can be seen, $CD4^-CD8^-$ cells from both patients were found to express the α/β form of the TCR. The intensity of α/β-TCR staining on these $CD4^-CD8^-$ cells did not differ significantly from that of control T cells (data not shown).

It is unlikely that contaminating $CD4^+$ or $CD8^+$ cells (whose surface markers were masked by the antibodies used in the purification process) are contributing to the results in Table II or Fig. 1 in that the monoclonal antibodies used in the negative selection process (OKT4 and OKT8) recognized different CD4 and CD8 epitopes from those used to assess $CD4^+$ and $CD8^+$ cell contamination of the purified $CD4^-CD8^-$ cells (Leu 2 and Leu 3). In addition, control experiments were performed in which normal T cells were pretreated with OKT4 and OKT8 before staining with Leu 2 and Leu 3. When examined by flow cytometry, the pretreated cells exhibited the same intensity and percentage of $CD4^+$ and $CD8^+$ staining as did cells that were not pretreated (data not shown). Finally, if $CD4^+$ or $CD8^+$ cells (whose surface markers were masked by OKT4 or OKT8 antibodies) were present in the $CD4^-CD8^-$ preparation they would be detected by the anti-mouse IgG control stain used in Fig. 1.

In summary, the $CD4^-CD8^-$ T cells from these patients express surface markers characteristic of mature peripheral blood T cells (CD3, CD2, CD5), express the α/β form of the TCR, and do not express surface markers characteristic of immature thymocytes (CD1) or NK cells (CD16, CD56).

Analysis of lymph node biopsy. Patient 1 underwent cervical and inguinal lymph node biopsy and both lymph nodes showed similar pathologic changes. The paracortex was expanded by a mixed population of lymphocytes, immunoblasts, and plasma cells. A follicular hyperplasia was present as well (Fig. 2).

By frozen section immunohistochemistry, the B cells were largely confined to the follicles and were polyclonal, as shown by staining with anti-λ and anti-κ antibodies. The paracortex

Table I. Lymphocyte Penotypic Profile and Serum Immunoglobulin Levels

	Peripheral blood			Lymph node‡	
	Patient 1	Patient 2	Normal Range*	Patient 1	Patient 2
Lymphocyte surface markers [*percent positive (absolute number μl)*]					
T cell markers					
CD 3 (Leu 4)	56 (8,833)	56 (2,880)	73±9 (1,484±499)	83	60
CD 2 (Leu 5)	56 (8,833)	52 (2,694)	80±6.7 (1,686±520)	63	57
CD 4 (Leu 3)	11 (1,717)	18 (927)	45±8.7 (909±266)	28	15
CD 8 (Leu 2)	10.7 (1,670)	17 (876)	29±7.3 (606±270)	12	13
CD 4/CD 45RA (2H4)	6.6 (1,030)	4.6 (237)	25±8.5 (484±169)	ND	ND
B cell marker					
CD 20 (Leu 16, B1)	41 (6,414)	47 (2,442)	11.8±4.8 (245±153)	6	33
NK cell markers					
CD 16 (Leu 11)	2.4 (375)	1.5 (77)	12.3±8.3 (263±204)	ND	1
CD 56 (NKH-1)	5.5 (858)	6.3 (325)	18.3±8.7 (376±213)	0	1
CD 57 (Leu 7)	36.5 (5,696)	20 (1,036)	1.3±8 (232±195)	0	21
Serum immunoglobulins *(g/liter)*					
IgG	1.78	2.3	1.6–0.65		
IgA	0.52	0.50	0.415–0.065		
IgM	0.57	0.24	0.320–0.050		

* For lymphocyte surface markers, mean±SD. ‡ Percent positive, as determined by flow cytometry.

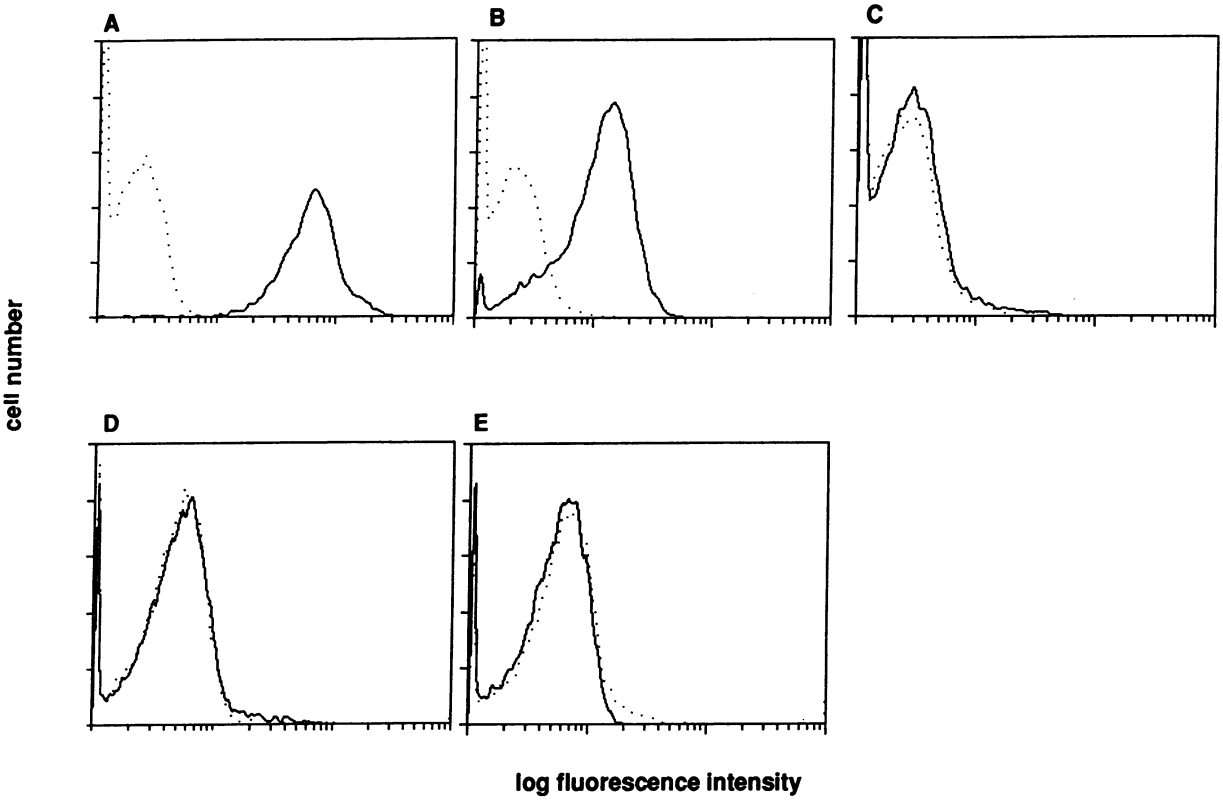

Figure 1. Phenotypic analysis of $CD4^-CD8^-$ T cells. Purified $CD4^-CD8^-$ T cells from the peripheral blood of patient 1 were stained with the following FITC-conjugated monoclonal antibodies and analyzed by flow cytometry: (*A*) Leu 4 (anti-CD3); (*B*) WT31 (anti-TCRα/β); (*C*) TCRδ-1 (anti-TCRγ/δ); (*D*) Leu 3 (anti-CD4); (*E*) Leu 2 (anti-CD8). The dotted line represents staining with a control antibody (FITC-conjugated goat anti-mouse IgG).

was heavily infiltrated by mature T cells with a $CD3^+CD4^-CD8^-$phenotype, identical to that seen in the peripheral blood (Fig. 2). These cells were positive for the activation-associated antigens CD38 and HLA-DR, but negative for CD30 and IL2-R. Many of the paracortical cells were positive for Ki-67, indicating active proliferation (20). In paraffin sections, the paracortical cells were strongly $CD43^+$, but $CD45RO^-$, suggestive of a "naive" or "virgin" phenotype. By contrast, the T cells found within the germinal centers were both $CD43^+$ and $CD45RO^+$, a phenotype characteristic of memory T cells.

Patient 2 underwent an occipital lymph node biopsy which revealed pathological and immunohistochemical findings similar to those of patient 1, but the abnormal phenotype ($CD3^+CD4^-CD8$) was expressed by only a portion of the paracortical T cells (data not shown). In patient 1, > 95% of T cells were $CD3^+CD4^-CD8$ whereas in patient 2 ~ 30% of T cells expressed this phenotype. The results of flow cytometry analysis of lymph node suspensions from both patients are listed in Table I. Staining of lymph node sections with monoclonal antibodies to EBNA-2 and LMP-1, two antigens expressed during latent infection in EBV transformed cells (13), was negative in both patients (data not shown).

Table II. Surface Phenotype of Peripheral Blood $CD4^-8^-$ T Cells

Antibody	Percentage of cells positive	
	Patient 1	Patient 2
CD 1 (Leu 6)	<5	ND
CD 3 (Leu 4)	>95	>95
CD 5 (Leu 1)	94	>95
CD 16 (Leu 11)	<5	ND
CD 56 (NKH-1)	<5	ND
CD 25 (IL 2R)	<5	<5
CD 45RA (2H4)	90	ND
TCR α/β (WT31)	>95	>95
TCR γ/δ (TCRδ1)	<5	<5

Activation requirements for $CD4^-CD8^-$ T cells: To study the activation requirements of the TCRα/β $CD4^-CD8^-$ T cell population, the cells were purified and exposed to a variety of activation stimuli. Table III compares the proliferative responses of the TCRα/β $CD4^-CD8^-$ T cells from these patients with those of control T cells. TCRα/β $CD4^-CD8^-$ T cells from both patients exhibited a deficient proliferative response to PHA/PMA, immobilized anti-CD3, and anti-CD2. In contrast, control T cells exhibited substantial proliferation to all three stimuli. The unseparated T cells from both patients consistently exhibited a degree of proliferation that was intermediate between that of the control T cells and the TCRα/β $CD4^-CD8^-$ T cells, suggesting that $CD4^+$ and $CD8^+$ T cells from these patients have a normal capacity to respond to these stimuli (data not shown).

The addition of recombinant IL-2 (rIL-2) to TCRα/β $CD4^-CD8^-$ cells stimulated with either anti-CD3 or anti-CD2 antibodies resulted in substantial augmentation of proliferation. This finding indicates that while these mitogenic antibodies are unable to induce full cell activation, they are able to

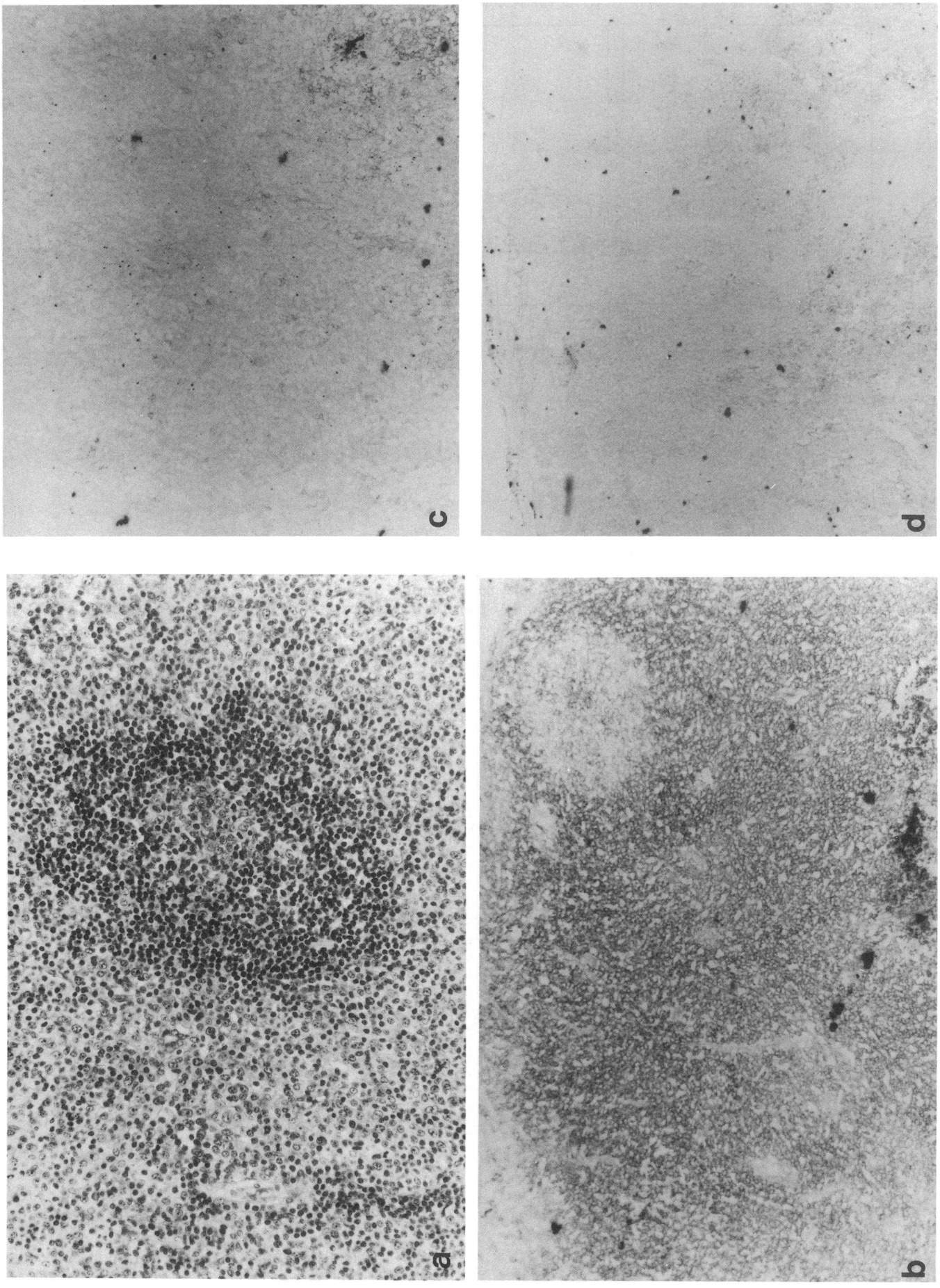
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Table III. Proliferative Response of CD4⁻8⁻ T Cells

Activation stimulus	CD4⁻8⁻ (patient 1)	CD4⁻8⁻ (patient 2)	Total T (normal controls)
Unstimulated	748±85	213±18	245±22
PHA/PMA	4,798±114	9,655±1,952	86,231±5,311
Anti-CD3	1,920±312	11,722±2,071	218,513±3,305
Anti-CD3 + rIL-2*	32,630±1,693	35,738±1,659	ND
Anti-CD3 + anti-CD28	22,663±2,050	313,057±668	109,616±16,508
Anti-CD2	328±86	2,573±682	188,436±12,664
Anti-CD2 + rIL-2	14,625±846	24,820±1,109	175,173±16,478
Anti-CD2 + anti-CD28	10,155±1,470	28,679±1,617	170,366±15,836

* rIL-2 used at 100 U/ml.

induce at least some degree of IL-2 receptor expression by these cells.

Anti-CD28 monoclonal antibodies are known to co-stimulate T cell proliferation induced by a number of stimuli (21). To determine if the deficient proliferation of these cells could be augmented via the CD28 activation pathway, purified TCRα/β CD4⁻CD8⁻ T cells were treated with either anti-CD3 or anti-CD2 antibodies (at optimal concentrations) in combination with the anti-CD28 antibody 9.3. Co-stimulation with anti-CD28 substantially augmented both anti-CD3 and anti-CD2-induced T cell proliferation (Table III), suggesting that the CD28 T cell activation pathway is functionally intact in these cells.

Production of IL-2 and interferon-γ by CD4⁻CD8⁻ T cells. To correlate lymphokine production with proliferation, levels of IL-2 and interferon-γ were determined in supernatants from cultures of purified TCRα/β CD4⁻CD8⁻ T cells. As would be predicted from the proliferation data, TCRα/β CD4⁻CD8⁻ T cells stimulated with immobilized anti-CD3 did not secrete significant amounts of IL-2 (Table IV). When these cells were co-stimulated with anti-CD28, a significant increase in IL-2 production was observed. However, the amount of IL-2 produced when TCRα/β CD4⁻CD8⁻ T cells were co-stimulated with anti-CD28 was considerably less than that produced by normal T cells stimulated in the same manner (Table IV). As with IL-2, stimulation of TCRα/β CD4⁻CD8⁻ T cells with immobilized anti-CD3 failed to induce significant interferon-γ production, while co-stimulation with anti-CD28 was able to induce interferon-γ secretion. Taken together, these findings provide further evidence that the CD3 activation pathway is deficient in the TCRα/β CD4⁻CD8⁻ T cells, while the CD28 pathway is at least partially intact.

Discussion

In this report we describe two young girls with a novel syndrome characterized by hypergammaglobulinemia, autoantibody production, lymphocytosis, hepato-splenomegaly, and prominent lymphadenopathy. A unique characteristic of their disorder is the profound expansion of an unusual T cell subset which expresses the α/β form of the TCR but does not express either the CD4 or CD8 surface markers (i.e., the cells are TCRα/β CD4⁻CD8⁻). These T cells are polyclonal and present in large numbers in the peripheral blood and lymph nodes of the two patients.

The clinical and immunopathological features of these two cases bear a resemblance to a lymphoproliferative disease seen in certain strains of mice which carry either the *lpr* or *gld* genes. These autosomal recessive genes induce in mice a syndrome characterized by progressive accumulation of nonmalignant TCRα/β CD4⁻CD8⁻ T cells in peripheral lymphoid tissue, hypergammaglobulinemia, autoantibody production, glomerulonephritis, and other autoimmune phenomena (6). The phenotypic expression of the *lpr* and *gld* genes is strongly influenced by background genes. This is illustrated by the variation in the degree of lymphoid hyperplasia and in the spectra of autoantibodies seen in different inbred strains of mice homozygous for *lpr* (22).

Like *lpr/gld* mice, our two patients exhibit autoimmunity occurring in association with the abnormal accumulation of TCRα/β CD4⁻CD8⁻ T cells. However, the disease in our two patients differs from that in *lpr/gld* mice in several respects. First, the types of autoantibodies produced by *lpr/gld* mice (anti-dsDNA, anti-Sm, anti-immunoglobulin) differ from those produced by our two patients (anti-nuclear and anti-RBC). Second, glomerulonephritis is a prominant feature of *lpr/gld* disease but occurred in only one of our patients. Third, severe autoimmune hemolytic anemia, which is present in one of our patients, is not a feature of *lpr/gld* disease. Despite these differences, which may be due to the presence of differing background genes, it seems reasonable to suggest that these patients are the human counterparts of *lpr/gld* mice.

The exact role played by the expanded population of TCRα/β CD4⁻CD8⁻ T cells in the development of autoimmune disease in *lpr* and *gld* mice is unknown. On the one hand, a causal relationship is suggested by the fact that interventions which prevent the accumulation of TCRα/β CD4⁻CD8⁻ T cells (i.e., neonatal thymectomy or treatment with anti-CD4, cyclosporin A, or IL-2) also prevent or lessen the autoimmune disease (23–26). On the other hand, these interventions also effect CD4⁺ and CD8⁺ T cell number and function. Furthermore, it has been shown that lymphoproliferative disease and autoantibody production in *lpr* mice can be dissociated (25, 27). Treatment of *lpr* mice with cyclosporin A results in a profound decrease in the accumulation of TCRα/β CD4⁻CD8⁻ T cells along with marked improvement in immune-mediated pathology (arthritis, glomerulonephritis). However, such treatment does not effect levels of total immunoglobulin, anti-DNA antibodies, or circulating immune complexes (25). Similarly, accumulation of abnormal TCRα/β CD4⁻CD8⁻ T cells is completely prevented in *lpr* mice transgenic for a rearranged TCR gene whereas hypergammaglobulinemia and autoantibody production are unaffected (27).

Figure 2. Histologic and immunohistochemical studies of the lymph node from patient 1. (*A*) The lymph node shows marked expansion of the paracortex by immunoblasts, lymphocytes, and plasma cells. Residual hyperplastic follicles are present (H&E; original magnification ×66). (*B*) A frozen section stained for CD3 (Leu 4) demonstrates strong staining of nearly all of the paracortical cells. The follicle is negative. (*C* and *D*) In parallel frozen sections the paracortical cells are negative for CD4 (*C*) and CD8 (*D*). Scattered macrophages are weakly CD4 positive. For panels *B*, *C*, and *D*, the ABC immunoperoxidase technique was used with a methyl green counterstain (original magnification ×33).

Table IV. Lymphokine Production by CD4⁻8⁻ T Cells

Stimulus	IL-2			Interferon-γ		
	CD4⁻8⁻		Total T (control)	CD4⁻8⁻		Total T (control)
	Patient 1	Patient 2		Patient 1	Patient 2	
	U/ml					
Unstimulated	5	0	6	0	0	2
Anti-CD3	8	0.2	54	0	0	32
Anti-CD3 + anti-CD28	100	9.4	2,765	56	9	73

Thus, the precise relationship between the expansion of the TCRα/β CD4⁻CD8⁻ T cells and the development of autoimmunity in *lpr/gld* mice remains to be defined.

TCRα/β CD4⁻CD8⁻ T cells are present in normal humans where they constitute a minor (< 1%) subpopulation of thymus and peripheral blood cells (3, 5). Recently, such cells have also been identified in normal skin (28). Because these cells are present in extremely low numbers in normal humans, the only information about their function comes from the study of TCRα/β CD4⁻CD8⁻ T cell lines derived from normal individuals (29) and a single patient with severe combined immunodeficiency (30). These cell lines were shown to exhibit cytotoxic activity, produce interferon-γ, and tumor necrosis factor.

The large percentage of TCRα/β CD4⁻CD8⁻ T cells in the peripheral blood of the patients reported here provided us with a unique opportunity to isolate a sufficient number of cells to assess their phenotypic and functional properties. In studies of cell phenotype, we found that the TCRα/β CD4⁻CD8⁻ T cells expressed surface proteins typically found on mature T cells (i.e., CD2, CD3, CD5) but did not express the thymocyte marker CD1, nor surface markers characteristic of NK cells (CD16, CD56). This surface phenotype is analgous to that of *lpr/gld* CD4⁻CD8⁻ T cells except that the murine CD4⁻CD8⁻ cells do not express CD2 (31).

In studies of cell activation, we found that the TCRα/β CD4⁻CD8⁻ T cells exhibited a defect in the CD3/TCR-associated activation pathway. Stimulation with either PHA or mitogenic anti-CD3 antibody resulted in markedly deficient proliferation and lymphokine production. A similar defect in the TCR-mediated activation pathway is seen in murine *lpr* TCRα/β CD4⁻CD8⁻ T cells (32). In further studies, we examined the capacity of the TCRα/β CD4⁻CD8⁻ T cells to be stimulated via the CD2 activation pathway. Using mitogenic anti-CD2 antibodies we found markedly deficient proliferation, indicating that this T cell activation pathway is also abnormal.

Recently, an alternate T cell activation pathway involving the CD28 antigen has been described. Certain anti-CD28 antibodies augment T cell proliferation to suboptimal doses of anti-CD3 or anti-CD2 monoclonal antibodies (21). Although antibodies to CD28 do not enhance proliferation of T cells which are stimulated with optimal doses of anti-CD3 or anti-CD2 monoclonal antibodies, they do induce a 5–50-fold increase in the levels of interferon-γ and IL-2 produced by activated normal T cells (21). Co-stimulation of our patient's TCRα/β CD4⁻CD8⁻ T cells with an anti-CD28 monoclonal antibody resulted in significant increases in both proliferation and lymphokine (IL-2, and interferon-γ) secretion. These findings indicate that, unlike the TCR and CD2 pathways, the CD28 activation pathway is functional in the TCRα/β CD4⁻CD8⁻ T cells.

The abnormal expansion of TCRα/β CD4⁻CD8⁻ cells in these two patients could be due to an intrinsic defect in T cell maturation, defective T cell processing in the thymus, or a combination of both. Along these lines, recent studies in TCR transgenic mice suggest a possible mechanism by which the abnormal accumulation of TCRα/β CD4⁻CD8⁻ T cells might lead to autoimmunity (33, 34). In these studies, transgenic mice were constructed which expressed a α/β TCR recognizing the male-specific antigen HY in the context of MHC class I. The vast majority of peripheral T cells found in the male transgenic mice expressed the TCR transgene (and were thus self-reactive), but were either CD4⁻CD8⁻ or expressed only low levels of CD8 (34). The results of these studies suggest that self-reactive T cells which fail to express CD4 or CD8 can escape deletion in the thymus. Based on these findings, it is possible to speculate that our two patients have an intrinsic defect in thymic T cell maturation which leads to the excessive production of TCRα/β CD4⁻8⁻ T cells. Because they do not express CD4 or CD8, self-reactive T cells within this CD4⁻8⁻ population might escape deletion in the thymus. These self-reactive TCRα/β CD4⁻CD8⁻ T cells could ultimately migrate to peripheral tissues, where they could undergo expansion in response to self-antigens and play a central role in the development of autoimmune disease.

In summary, we describe two girls in whom a novel syndrome evolved early in life. This syndrome is characterized by the occurrence of autoimmunity in the setting of massive lymphoid hyperplasia secondary to the accumulation of an unusual T cell subpopulation which is CD4⁻CD8⁻ and expresses the α/β form of the TCR. The clinical and immunopathogenic features of this case resemble the lymphoproliferative disease seen in *gld* and *lpr* mice and establish that excessive accumulation of TCRα/β CD4⁻CD8⁻ T cells in humans can also be associated with autoimmunity.

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Note added in proof. Since the submission of the manuscript for this article, Illum et al. (35) have reported a patient with lymphadenopathy, splenomegaly, and humoral immunodeficiency, who exhibited an expansion of TCRα/β $CD4^-CD8^-$ T cells. The phenotypic and functional properties of the double negative T cells from this patient were similar to those of our two patients. Their patient did not have autoimmune disease.

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