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T-cell receptor genes in autoimmune mice: T-cell subsets have unexpected T-cell receptor gene programs

(thymus cell antigen, θ/γ chain/thymus/ontogeny)

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ABSTRACT **Two unique cell subsets have been identified in the autoimmune-prone MRL/MP *lpr/lpr* and C3H/HeJ *gld/gld* murine strains that have the Lyt-2$^-$,L3T4$^-$,Thy-1$^+$, and Lyt-2$^-$,L3T4$^-$,Ia$^-$,Thy-1$^-$ phenotypes, respectively. We have now found that these cells express T-cell receptor proteins on their surface. Our observations further indicate that the expression of the Thy-1 antigen does not correlate with the expression of α-chain and β-chain T-cell receptor polypeptides. Interestingly, T-cell receptor γ-chain RNA expression may be influenced or correlate with Thy-1 molecular expression. These studies indicate unusual relationships of different cell-surface structures that may reflect unexpected developmental programs.**

The expression of T-cell receptor molecules as well as several related surface antigens is thought to occur in a defined pattern during T-cell development (1). In the current view of T-cell development, Thy-1 expression precedes the expression of other developmental antigens. Transcription of the γ and β chain of the T-cell receptor gene occurs subsequent to cell-surface expression of Thy-1 (1–4). Although γ-chain RNA expression precedes that of the β chain RNA in most T cells, the existence of T-cell subsets lacking γ-chain RNA expression in the periphery suggests that γ-chain expression is an optional step or a transient event occurring only at a certain stage of T-lymphocyte development (5). However, α-chain transcripts are often detected in relatively mature cells expressing L3T4 and/or Lyt-2 on the cell surface (2–4). It is thought that many T-cell differentiation events associated with the phenotypic changes described here occur primarily within the thymus (1–4, 6). After completing intrathymic development, T cells emigrate to the periphery to populate secondary lymphoid organs. In keeping with this scheme, abnormalities of the thymus gland have been suggested to affect T-cell development patterns (7, 8).

Certain murine strains seem to demonstrate an autoimmune syndrome that develops with age and also appears to be thymus dependent (9, 10). In one such strain, C3H/HeJ *gld/gld*, we have found two predominant and unique T-cell subsets: one is phenotypically Lyt-2$^-$,L3T4$^-$,Thy-1$^+$, which we refer to as "double negative," and the second is phenotypically Thy-1$^-$,Ia$^-$,Lyt-2$^-$,L3T4$^-$, which we term the "null subset." Both subsets are observed in the periphery of two autoimmune-prone mice: MRL/MP *lpr/lpr* and C3H/HeJ *gld/gld* (10). For the studies undertaken here, we have specifically used aged *gld/gld* mice because the T-cell subset abnormalities, although present by 4 weeks after birth, become exceptionally prominent after 3 months of age. It is also noteworthy that aged athymic mice have been shown to be deficient in normal T lymphocytes but contain significant numbers of L3T4$^-$,Lyt-2$^-$,Thy-1$^+$ cells in their secondary lymphoid organs (8). Based upon these T-cell abnormalities, we have suggested that C3H/HeJ *gld/gld* mice have a thymus defect that promotes the accumulation of immature T cells (7). In the study reported here, we have evaluated different T-cell subsets from adult *gld/gld* mice in terms of T-cell receptor gene expression patterns. Unexpectedly, we observed a dissociation between Thy-1 and T-cell receptor α/β-chain gene expression and an association with γ-chain transcription.

MATERIALS AND METHODS

Mice. C3H/HeJ *gld/gld* mice were obtained from The Jackson Laboratory. The mice were bred and maintained in our colony at the University of Pennsylvania and used when >3 months of age. C3H/HeJ +/+, BALB/cJ mice were purchased from The Jackson Laboratory and were used when 6–12 weeks of age.

Cell Preparation. T cells of normal and *gld/gld* strain were enriched by passage through nylon-wool columns, and some of them were treated with lytic amounts of monoclonal anti-Lyt-2 (3-155) (11) antibody and anti-L3T4 (GK-1.5) (12) antibody and complement. To obtain null cells, the total cells were treated with monoclonal anti-Thy-1 (HO13.4) (13), anti-Ia (14.4.4S) (14), anti-Lyt-2, and anti-L3T4 antibodies and complement.

Flow Cytometry Analysis. For fluorescent staining, 1×10^6 cells were incubated in 100 μl of phosphate-buffered saline (P_i/NaCl, pH 7.2) supplemented with 0.5% bovine serum albumin and 0.1% sodium azide with various monoclonal antibodies for 20 min at 4°C. After one wash, the pellets were incubated with 50 μl of fluorescein isothiocyanate-coupled anti-rat κ-chain monoclonal antibody (Becton Dickinson) at 4°C for an additional 20 min. After three washes, the cells were analyzed using fluorescence-activated cell sorter FACS IV (Becton Dickinson).

RNA Blot Hybridization. Total cellular RNA was extracted from enriched T cells in the presence of guanidine thiocyanate (15). Total RNA was denatured with glyoxal, subjected to electrophoresis through a 1% agarose gel, transferred to nitrocellulose (16) and, after prehybridization, hybridized with the labeled probe in 50% formamide containing 5× NaCl/Cit (1× = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0), 50 mM sodium phosphate (pH 6.5), 250 μg of sheared and denatured salmon sperm DNA per ml, 0.5% $NaDodSO_4$, and 10% dextran sulfate for 30 hr at 37°C. The filter was washed in 2× NaCl/Cit containing 0.1% $NaDodSO_4$ four times at room temperature for 15 min each and then washed with 0.1× NaCl/Cit containing 0.1% $NaDodSO_4$ twice for 30 min each at 50°C.

The probes were labeled by the oligolabeling method (17) to obtain high specific activities (3×10^8 cpm per μg).

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RESULTS

Surface Expression of Thy-1, Lyt-2, and L3T4 Molecules and of the T-cell Receptor. The cell-surface expression and RNA levels of several proteins were evaluated in double-negative and null-cell lymphoid subsets of *gld/gld* mice. The percentage of T cells in the lymph nodes of 6-month-old C3H/HeJ *gld/gld* mice is significantly higher than in normal C3H/HeJ counterparts (7, 9, 10). T cells isolated from lymph nodes were enriched by passage through nylon-wool columns and then were treated with lytic amounts of monoclonal anti-Lyt-2 antibody, anti-L3T4 antibody, and complement. To obtain null cells, the total cells were treated with monoclonal anti-Thy-1, anti-Ia, anti-Lyt-2, and anti-L3T4 antibodies and complement instead of nylon-wool column passage.

The expression of Thy-1, Lyt-2, L3T4, and the T-cell receptor (KJ16 monoclonal antibody-reactive molecules) on the cell surface of the total population or subsets of T cells obtained from the lymph node of C3H/HeJ *gld/gld* strain was analyzed by using flow cytometry. These patterns were compared with T cells derived from the lymph node of the normal C3H/HeJ strain (Fig. 1). It was apparent that the population of C3H/HeJ *gld/gld* lymphocytes includes a significant amount of Thy-1$^-$ cells. The number of C3H/HeJ *gld/gld* Lyt-2$^+$ and/or L3T4$^+$ cells was reduced when compared with lymphocytes of the same phenotype found in normal C3H/HeJ lymph nodes. This lower number of Lyt-2- or L3T4-expressing cells in *gld* reflects the dilution by a large number of double-negative cells and a subset of "null cells." However, the number of the T-cell receptor (KJ16$^+$)-bearing cells in the *gld/gld* strain was only slightly less than that seen in the normal strain.

Detailed analysis of the double-negative cells showed that this subset also expressed T-cell receptor molecules on the cell surface (Fig. 1). Previously, double-negative cells were considered to be immature lymphoid cells. However, recent data suggest that double-negative T cells in the normal mouse express γ-chain-bearing T-cell receptor molecules on the cell surface (19, 20). Our studies indicate that double-negative cells in the *gld* strain are not completely immature in terms of T-cell development inasmuch as they expressed the T-cell receptor, a phenotype characteristic of cells that have undergone some level of programmed differentiation. Interestingly, null cells also expressed T-cell receptor epitopes detectable by the monoclonal antibody KJ16. Therefore, some parts of null cells are more appropriately considered a subset of T cells than belonging to a non-T/non-B-cell lineage within *gld/gld* mice.

RNA Expression of Genes Encoding L3T4, Lyt-2, and Thy-1. It is conceivable that the monoclonal antibodies we had used in these staining analyses could not detect altered molecules. Therefore, we evaluated whether these cell subsets lacking cell-surface L3T4, Lyt-2, or Thy-1 might nevertheless contain

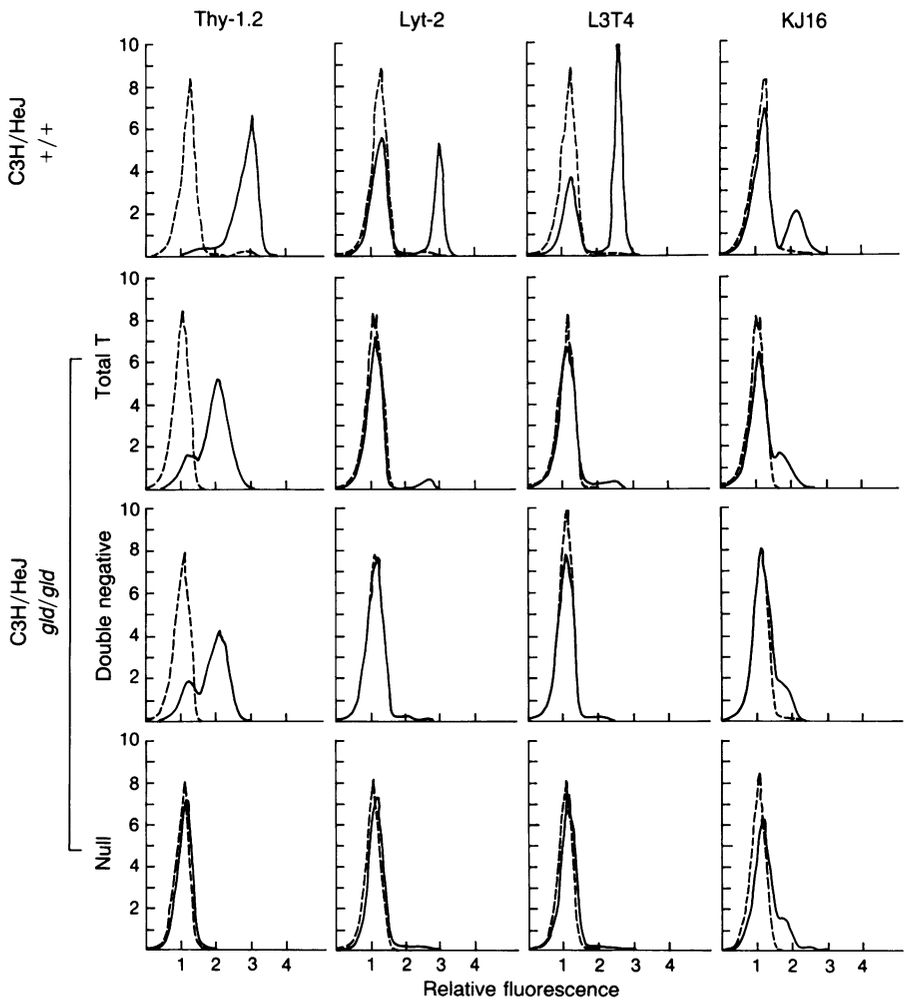

FIG. 1. Flow cytometric analysis of peripheral T cells obtained from C3H/HeJ normal (+/+) or *gld/gld* strain. Each cell subset was prepared as described and stained with monoclonal anti-Thy-1 (directly conjugated with fluorescein isothiocyanate) (HO13.4) (13), anti-Lyt-2 (3-155) (11), anti-L3T4 (GK1.5) (12), or anti-T-cell-receptor (KJ16-133) (18) antibody. The negative control FACS profile for Thy-1 represents cells without primary antibody. For other antibodies, the cells were stained with secondary antibody alone as a negative control. —, Profile of antibody staining; ---, negative control.

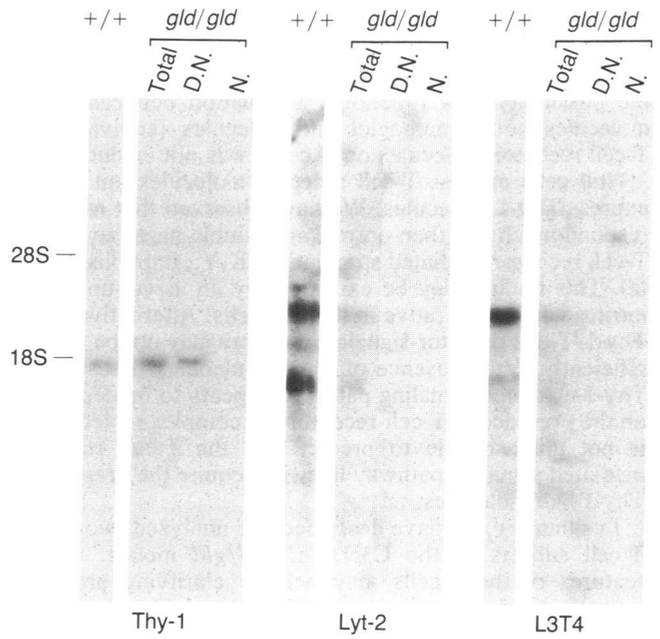

FIG. 2. Blot-hybridization analysis of genes encoding Thy-1, Lyt-2, and L3T4 in T cells obtained from normal-strain mice (+/+) and in T-cell subsets from the *gld/gld*-strain mice: total T cells (Total), double-negative T cells (D.N.), and null cells (N.). The same blotted filter was hybridized with ^{32}P-labeled Thy-1 (pT64) (21), Lyt-2 (pLy2-7B) (23), and L3T4 (p3c) (22) cDNA clones.

the RNA for those molecules. The cell subsets were prepared as described, and RNA was purified and analyzed by blot hybridization. The blots (Fig. 2) were probed with ^{32}P-labeled Thy-1 (21), L3T4 (22), and Lyt-2 (23) cDNA probes. These studies revealed that double-negative cells did not express L3T4, Lyt-2, or Thy-1 RNA at the detectable level. Both the double-negative and null-cell subsets expressed monoclonal antibody KJ16-reactive T-cell receptors on the cell surface without coexpression of L3T4 or Lyt-2 structures.

RNA Expression of T-Cell Receptor Genes. To analyze these cell subsets in terms of the types of T-cell receptor chains expressed at the RNA level, the same membranes were rehybridized with ^{32}P-labeled T-cell receptor α-, β-, and γ-chain cDNA constant region probes (Fig. 3). We have previously shown that the T-cell receptor α- and β-chain RNA level is enhanced in lymph node-residing bulk T cells obtained from the C3H/HeJ *gld/gld* strain (7). The present studies revealed that isolated T-cell subsets also demonstrated enhanced α- and β-chain RNA levels.

Interestingly, hybridization of RNA blots with the T-cell receptor γ-chain gene probe revealed that γ-chain gene expression was barely detected in null cells when compared with that of double-negative cells. Since the level of the γ-chain gene expression is much lower than many other expressed genes, it is often difficult to distinguish the signals from the background noise. The film for the blot hybridization to detect expression of the T-cell receptor γ-chain gene was exposed about 10 times longer than the corresponding blots for α- and β-chain gene expression to obtain similar intensities of signals. Repeated blot hybridizations consistently demonstrated similar results. Therefore, we concluded that null cells do not express the T-cell receptor γ-chain gene at a detectable level on RNA blots.

RNA blots with a T3 probe revealed enhanced expression of this molecule at the RNA level in all T-cell subsets of the *gld/gld* strain. This was in agreement with previous observations of cell-surface coexpression of T3 and T-cell receptor α and β chains (28). Our results suggest that cell-surface expression of T3 and T-cell receptor chains precedes or can be disassociated from expression of Lyt-2 or L3T4 in *gld/gld* T cells.

DISCUSSION

The autoimmune-prone adult C3H/HeJ *gld/gld* mouse accumulates large numbers of T lymphocytes in the secondary lymphoid organs. The majority of the T cells belong to two

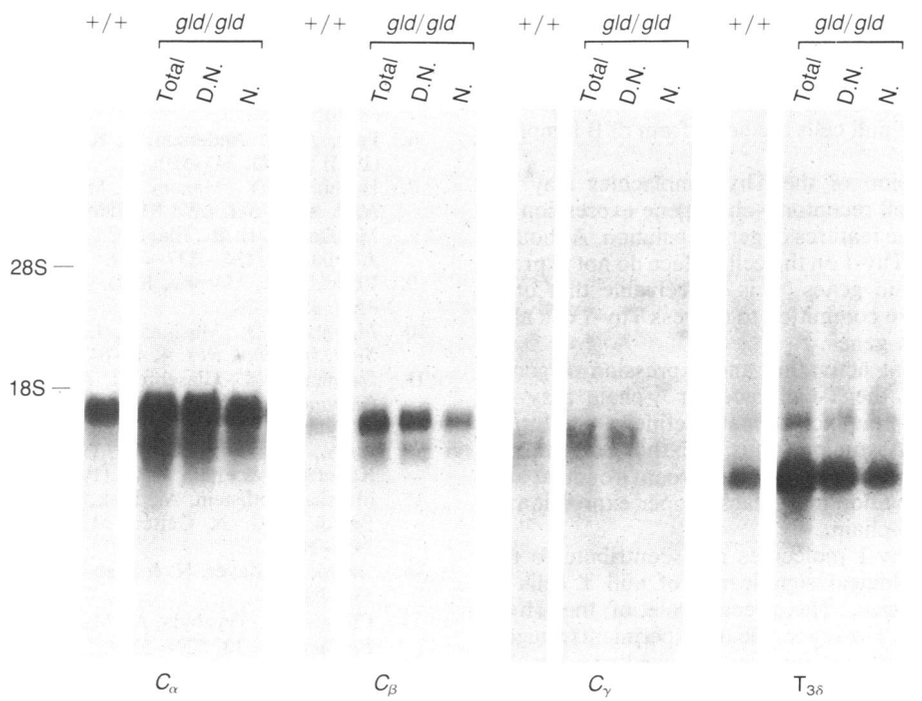

FIG. 3. Blot-hybridization analysis of the T-cell receptor α-, β-, γ-, and δ-chain genes in T cells obtained from normal-strain (+/+) mice and in T-cell subsets from the *gld/gld*-strain mice (total, D.N., and N. as in Fig. 2). Total RNA (10 μg) was analyzed as in Fig. 2. Probes used were ^{32}P-labeled pHDS58, containing the constant region of the α-chain gene, for the T-cell receptor α-chain gene (C_α) (24); pUC25 for the T-cell receptor β-chain gene (C_β) (25); pHDS205, containing the constant region of the γ-chain, for the T-cell receptor γ-chain gene (C_γ) (26); and pPGBC-9 for the T3 δ-chain gene (T3δ) (27). The blot for the T-cell receptor γ-chain gene was exposed 10 times longer than the others.

phenotypically distinct subsets, which include null (Thy-1$^-$, Lyt-2$^-$, L3T4$^-$, and T-cell receptor positive) and double-negative cells (Thy-1$^+$, Lyt-2$^-$, L3T4$^-$, and T-cell receptor positive). In this study, we have observed that each subset has a different pattern of T-cell receptor gene expression.

The Thy-1 molecule has been useful as a marker of T-cell lineage. However Thy-1 expression is not restricted to T lymphocytes. Neurons, fibroblasts, and myoepithelial cells may also express this protein (21). Low amounts of Thy-1 antigen also have been observed on so-called pre-pre-B-cells (29). Recent evidence suggests that Thy-1 structures may act as signal-transduction molecules (30, 31). The L3T4 antigen may also be expressed in some form in neuronal tissues (32). These observations suggest that L3T4 and Thy-1 may have some as-yet-undefined function in nonlymphoid tissues. Certainly, the mere presence of these proteins on nonlymphoid cells questions the validity of referring to them as tissue specific. Therefore, it is not surprising that Thy-1, Lyt-2, and L3T4 gene expression can be dissociated from the regulation of T-cell-specific genes such as the T-cell receptor, as observed in the studies described here.

It is possible that T-cell receptor γ-chain RNA expression is variable in certain subsets of T cells. Alternatively γ-chain expression may be very transient in some subsets but persist in others. The absence of T-cell receptor γ-chain expression in the null-cell subset may also be explained in other ways. First, null cells might represent an oligoclonal expansion of a subset of T cells that does not express the T-cell receptor γ-chain gene. However, Southern blots of the null cells failed to reveal limited major rearranged bands of the T-cell receptor β-chain gene (data not shown), which might characterize oligoclonal populations. Rather, the pattern of T-cell receptor gene rearrangement in the null-cell subset was varied, implying that bulk null cells are heterogeneous with respect to T-cell β-chain genes.

Second, the immature phenotype of the null-cell subset might suggest that this cell type represents a developmental stage preceding divergence of T- and B-cell-specific lineages. However, the null cells express T-cell receptor α- and β-chain genes at both the RNA level and on the cell surface. Furthermore, immunoglobulin molecules were not detected on the cell surface by cytofluorometric analysis or in the cytoplasm by immunostaining (data not shown), excluding the consideration that null cells are some form of B lymphocytes.

Third, the expression of the Thy-1 molecules may be coordinated with T-cell receptor γ-chain gene expression as a consequence of some features of gene regulation. Although many T cells bearing Thy-1 on the cell surface do not express T-cell receptor γ-chain genes, it is conceivable that only lymphoid cells that are committed to express Thy-1 can also express γ-chain RNA genes.

Fourth, independent activation and expression of genes encoding Thy-1 and the T-cell receptor γ-chain may be coincidentally programmed to occur at a defined stage during T-cell development. Assuming this is true, those cells entering an unsuitable or incorrect thymic microenvironment at a critical stage for activation might lack proper expression of both Thy-1 and the γ-chain.

The absence of Thy-1 molecules may contribute to the biological and pathological significance of null T cells in autoimmune-prone mice. The precise role of the Thy-1 molecule is unknown. However, recent experiments suggest that the Thy-1 molecule can function in signal transduction (30, 31). When Thy-1 molecules were introduced into B cells, monoclonal antibodies specific for Thy-1 could activate these cells (30). In nontransformed lymphocytes, Thy-1-mediated signal transduction seems to be influenced by elements associated with the T-cell receptor–T3 molecule complex (31). Both studies support the notion that Thy-1 structures may function as signal-transduction molecules. Although the former study demonstrated that the Thy-1-mediated signaling pathway was independent of the T-cell receptor–T3 complex, the possibility of a functional association between Thy-1 molecules and immunoglobulin molecules (equivalent to T-cell receptor molecules on T cells) was not excluded.

Null cells express T-cell receptor molecules but do not express Thy-1 molecules. We have observed that null cells respond much less than normal and double-negative cells to T-cell receptor-mediated stimulation (K.Y., unpublished data). This finding may be explained by an as-yet-undefined intrinsic defect operative in the null cells. Alternatively, the Thy-1/T-cell receptor-signaling pathway may not be utilized efficiently in the absence of Thy-1 molecules. Because the Thy-1-mediated signaling pathway appears to be dependent on the presence of T-cell-receptor–T3 complex molecules, it is not unreasonable to predict that the T-cell receptor-mediated signaling pathway likewise require the presence of Thy-1-like molecules.

In summary, we have described and analyzed two unique T-cell subsets of the C3H/HeJ *gld/gld* mouse. Certain features of these cells may help in clarifying processes influencing the expression of Thy-1 and T-cell receptor γ-chain genes. The isolation and cloning of these novel cells will provide unique opportunities to analyze biological activities of the T-cell receptor in the absence of other structures thought to be relevant to T-cell recognition of antigen.

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