Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 7865–7869, October 1986
Immunology

T-cell antigen-receptor genes in autoimmune mice

(T-cell differentiation/thymus selection/DNA rearrangement/autoimmune disease)

YASUHIRO HASHIMOTO*†, ALLAN M. MAXAM*‡, AND MARK I. GREENE†

*Dana–Farber Cancer Institute and ‡Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115; and †Division of Immunology, Department of Pathology, University of Pennsylvania, Philadelphia, PA 19104

Communicated by James M. Sprague, June 9, 1986

ABSTRACT **The developmental patterns of rearrangement and expression of the T-cell antigen-receptor genes are precisely regulated during T-cell differentiation and education. The β- and γ-subunit RNAs of the T-cell receptor are abundantly expressed in immature thymocytes. In mature thymocytes the α- and β-subunit RNAs are preferentially expressed, whereas there is minimal expression of the γ RNA. Although aspects of the pattern of known organization and rearrangement of the T-cell receptor gene in the thymus have been studied and the concept of a thymus selection process generally has been accepted, the cellular and molecular basis of thymus education remains obscure. Certain strains of mice with predilections for autoimmunity demonstrate T-cell developmental abnormalities. This is especially true for the *lpr/lpr* or *gld/gld* genotypes, in which the major population of peripheral T cells is developmentally disturbed. We have studied the development, expression, and rearrangement of T-cell receptor genes in the C3H/HeJ *gld/gld* mouse. Our results indicate a high level of expression of the β and α RNAs in C3H/HeJ *gld/gld* T cells residing in the periphery. In addition, the β-subunit gene of *gld/gld* peripheral T cells undergoes more rearrangements than does its normal C3H/HeJ T-cell counterparts. We speculate that this rearrangement pattern and high level of T-cell receptor mRNA reflects an abnormality or deficit in a thymus selection process that permits emigration of T cells with nonfunctionally rearranged T-cell receptor genes to secondary lymphoid organs. However, the normal level of γ-subunit RNA expression argues that γ-subunit gene rearrangements are distinct from processes related to α- and β-subunit selection.**

It is generally accepted that immunoglobulin and T-cell antigen receptors are developmentally and functionally related (1). The immunoglobulin proteins bind to a variety of foreign molecules. The general features of antibody molecules are that they consist of light and a heavy chains, both of which have a constant (C) and a variable (V) region. During B-cell development, the immunoglobulin heavy (H) chain variable (V_H), diversity (D_H), and joining (J_H)–constant (C_H) elements are brought together, followed by light (L) chain V_L, J_L–C_L rearrangement (2). The T-cell receptor molecules also recognize a variety of foreign antigens in the context of self molecules. The T-cell receptor molecules are composed of α and β chains, both of which have a C and a V region. Comparable organization of elements of the genes for the T-cell receptor and for immunoglobulin has been thought to indicate similarities of the processes of gene rearrangement active in these polymorphic gene systems (1). At an early stage of T-cell development, the γ-subunit and the α-subunit genes are rearranged and abundantly expressed in immature T cells of the Lyt-2$^-$ L3T4$^-$ phenotype located within the thymus. Rearrangement and expression of the α gene have been observed to occur at a later stage of T-cell development in the thymus. Therefore, functional T-cell development is apparently regulated within the thymus gland by processes affecting the organization and expression of the T-cell receptor (3–5). In this regard, it is generally accepted that the thymus gland provides a special but undefined milieu for T-cell development and selection of certain subpopulations of T cells. The process of T-cell development and mechanisms of T-cell selection at the molecular level are even more obscure. One approach to clarify these mechanisms involves the study of animals with defects in thymus function.

Certain autoimmune mice, with the *lpr/lpr* or *gld/gld* genotypes, develop massive lymphoproliferation and associated autoimmune processes that result in anti-DNA antibody formation and autoimmune kidney disease (6–9). The rodents accumulate a population of T cells in their periphery that are phenotypically immature: Lyt-2$^-$ L3T4$^-$ Thy-1$^+$. An age-related decrease in the frequency of alloantigen-specific cytolytic T-lymphocyte precursors (10–12) and concanavalin A-induced production of interleukin 2 (13–16) also have been observed in the *lpr* strain. In addition, neonatal thymectomy prevents development of autoimmune disease in the *lpr* strain of mouse (17). Although the autoimmune patterns of the *gld* strain are similar to the *lpr* strain, the mutations *gld* and *lpr* are not allelic (8).

We analyzed the rearrangement and expression of T-cell receptor genes in the C3H/HeJ *gld/gld* strain, which demonstrates age- and thymus-dependent autoimmune processes. The level of RNA expression of the T-cell receptor α subunit is strikingly high in T cells residing in the periphery and thymus, compared with the normal C3H/HeJ strain. Relatively high levels of expression of the β-subunit RNA were observed in T cells residing in the periphery. However, no significant differences of γ-subunit RNA expression were observed between the C3H/HeJ strain and C3H/HeJ *gld/gld* strain. In addition, the β-subunit gene of *gld/gld* peripheral T cells undergoes more extensive rearrangements than does its normal C3H/HeJ T-cell counterparts. Relevant to these studies are recent observations that demonstrate that certain immature B cells express high levels of nonfunctional immunoglobulin genes (18). By analogy, our data might indicate that high levels of immature T-cell receptor mRNA may reflect transcription of nonfunctional genes in T cells that are permitted to emigrate to the periphery by an imperfect thymus selection process operative in the *gld* strain.

MATERIALS AND METHODS

Mice. C3H/HeJ mice were obtained from The Jackson Laboratory and were bred in our animal facilities. C3H/HeJ *gld/gld* mice were initially provided by Alfred Steinberg

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: V, D, J, and C regions, variable diversity, joining, and constant regions; J_β and C_β, T-cell antigen receptor β-subunit gene *J* and *C* regions; V_H, D_H, and J_H, immunoglobulin heavy chain V, D, and C regions; kb, kilobase(s); MHC, major histocompatibility complex.

(National Institutes of Health) and then obtained from The Jackson Laboratory.

T-Cell Enrichment. Lymphoid cells obtained from the thymus and peripheral lymph nodes were passed through nylon-wool columns as described by Henry (19). After T-cell enrichment, aliquots of cells were routinely stained by using monoclonal anti-Thy-1.2 antibody (HO-13-4) (20) and analyzed by flow cytometry on a cytofluorograph (Becton Dickinson FACS IV).

Southern Blots. T cells from lymph node or thymus were enriched by a passage through nylon-wool columns. After enrichment, DNA was extracted and digested with the restriction enzymes, then separated on a 1% agarose gel, and transferred to GeneScreen*Plus* (New England Nuclear) by a modification of the method described by Southern (21). Transferred membranes were prehybridized and hybridized with the labeled probes in 50% (wt/vol) formamide containing 3× NaCl/Cit (1× NaCl/Cit = 0.15 M sodium chloride and 0.01 M sodium citrate), 5× Denhardt's solution (1× Denhardt's solution = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin), 50 mM Tris chloride (pH 7.5), 1 mM EDTA, 100 μg of sheared and denatured salmon sperm DNA per ml, 0.5% $NaDodSO_4$, and 10% (wt/vol) dextran sulfate for 20 hr at 37°C. After hybridization, the filter was washed at room temperature twice in 1× NaCl/Cit containing 0.1% $NaDodSO_4$ and at 50°C twice in 0.5× NaCl/Cit containing 0.1% $NaDodSO_4$ for 45 min. Both pUC25 (22) and pGXS (Fig. 1) probes were labeled by the oligo-labeling method (23) to high specific activities (3×10^8 cpm per μg) after the isolation of the inserts. Sizes of restriction fragments hybridizing to the probes were measured by using phage λ DNA restriction fragments of known length run in parallel on the same gel.

RNA Blot Hybridization. Total cellular RNA was extracted from enriched T cells in the presence of guanidine thiocyanate (24). Total RNA were denatured with glyoxal, subjected to eletrophoresis through a 1% (wt/vol) agarose gel, transferred to nitrocellulose (25), and, after prehybridization, hybridized with the labeled probe in 50% formamide containing 5× NaCl/Cit, 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.

RESULTS

Rearrangement of the T-Cell Receptor β-Subunit Gene. Initial experiments were designed to investigate the rearrangement of the T-cell receptor β-subunit gene in the peripheral T-cell population of C3H/HeJ *gld/gld* mice. The rearrangement of the T-cell receptor β gene within a heterogeneous population of T cells is readily detected on Southern blots by the disappearance of the bands corresponding to the T-cell receptor gene in the germ-line configuration, because individual T cells within the population often have differently sized rearranged bands that are hardly detectable on Southern blots. To estimate the extent of the T-cell receptor β gene rearrangement, we first used the *Hpa* I restriction endonuclease for DNA digestion and a cDNA $C_{\beta 2}$ region probe (pUC25), which cross-hybridizes to the $C_{\beta 1}$ region, for Southern blots. This combination shows two distinct bands in the germ-line configuration: an 11.6-kilobase (kb) band and a 6.1-kb band (Fig. 1). Since contamination of non-T-cells, in which T-cell receptor genes typically do not rearrange, may mislead the estimation of the extent of nonrearranged genes, T cells from lymph node or thymus were highly enriched by passage through nylon-wool columns. Examination of enriched T cells by anti-Thy-1.2 antibody staining and the flow cytometry revealed that contamination by non-T-cells was always <5%.

The same amount of the liver or lymph-node T cell-derived DNA obtained from control C3H/HeJ and C3H/HeJ *gld/gld* mice were digested with *Hpa* I. Southern analysis (Fig. 2*A*) revealed that the intensities of the 11.6-kb and 6.1-kb bands of DNA prepared from lymph-node T cells in the C3H/HeJ *gld/gld* strain mice were reduced when compared to the corresponding lymph-node T cell-derived DNA obtained from the C3H/HeJ strain.

To confirm this result and to control for trivial reasons that might increase or decrease the intensity of β-subunit gene bands on gels, we used another combination of restriction enzymes and probes to estimate the extent of rearrangement. The *Hin*dIII restriction enzyme for DNA digestion and a genomic J_β–$C_{\beta 2}$ region probe (pGXS), which extends 363 bases 3′ of $J_{\beta 2.6}$ to 3′ of $C_{\beta 2}$ exon 1 and which also cross-hybridizes to the $C_{\beta 1}$ region, were used (Fig. 1). This combination of the *Hin*dIII endonuclease and pGXS probe revealed four distinct bands in the germ-line configuration: 9.4-, 5.0-, 0.8-, and 2.9-kb bands (Fig. 1). When the T-cell

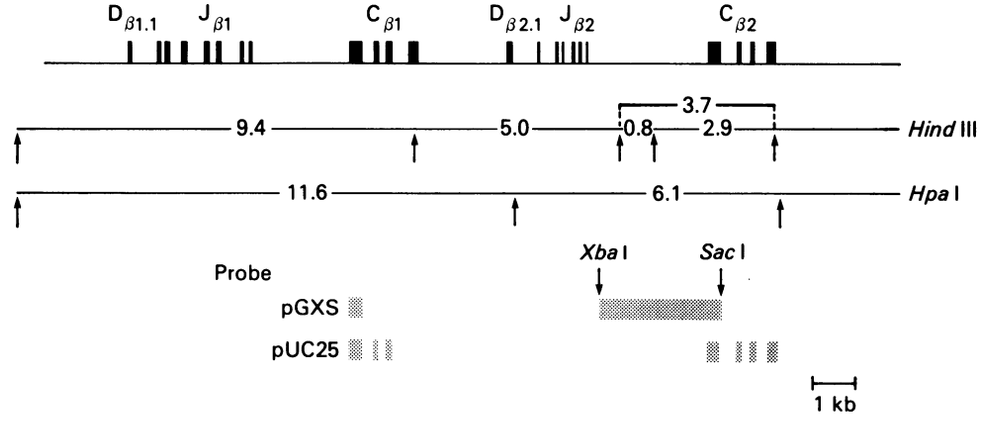

FIG. 1. Germ-line T-cell receptor β-subunit genes. Exons are depicted by vertical lines or black boxes above the horizontal line. Restriction enzyme cleavage sites for *Hin*dIII and *Hpa* I are indicated. The numbers in the lines of the restriction map show the length of expected bands on the gel. Segments hybridizing with the probes pGXS and pUC25 (22) are indicated by the stippled boxes. The clone pGXS hybridizes with the $J_{\beta 2}$–$C_{\beta 2}$ intron and $C_{\beta 2}$ exon 1 and also cross-hybridizes with $C_{\beta 1}$ exon 1. These clones were obtained by subcloning of the *Xba* I–*Sac* I fragment derived from the 4C4 BALB/c genomic clone into the Gemini 2 vector. The clone pUC25 hybridizes with the $C_{\beta 2}$ exon and cross-hybridizes with the $C_{\beta 1}$ exons 1, 2, and 3. These clones were obtained by subcloning *Eco*RI/*Eco*RI fragment derived from the B3C6 cDNA clone into pUC9 (22). Two *Hin*dIII cleavage sites in the $J_{\beta 2}$–$C_{\beta 2}$ intron were confirmed by sequencing of the pGXS clone.

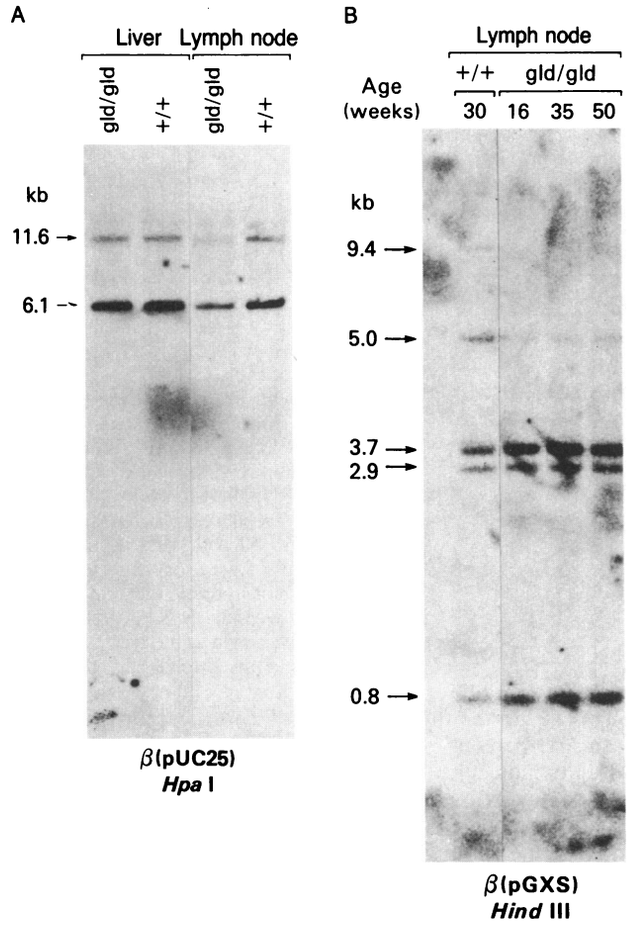

FIG. 2. Southern blot analysis of the T-cell receptor β-subunit gene in T cells and liver from C3H/HeJ +/+ strain (shown as +/+) and C3H/HeJ *gld/gld* strain (shown as *gld/gld*). T cells from lymph node were enriched by passage through nylon-wool columns. After enrichment, DNA was purified. Liver DNA was extracted as described by Maniatis *et al.* (26). (*A*) DNA (10 μg) was digested with 40 units of *Hpa* I restriction enzyme for 4 hr, separated on a 1% agarose gel, transferred to GeneScreen*Plus* (New England Nuclear), and, after prehybridization, hybridized with the ^{32}P-labeled pUC25 probe (Fig. 1). Conditions for hybridization have been described. The sizes of restriction fragments hybridizing to the probes were measured by using phage λ DNA restriction fragments of known length run in parallel on the same gel; 11.6- and 6.1-kb bands are in the germ-line configuration and containing $C_{\beta 1}$ and $C_{\beta 2}$, respectively. Since pUC25 hybridizes to the $C_{\beta 2}$ region more strongly than to the $C_{\beta 1}$ region, the intensity of the 6.1-kb bands is greater than that of the 11.6-kb bands. (*B*) DNA (10 μg) was digested with 40 units of *Hin*dIII for 4 hr. The same procedure as described above was followed except for the use of pGXS as the probe (Fig. 1). The age of mice is shown at the top of each column. Both pUC25 and pGXS probes were labeled by the oligo-labeling method to high specific activities (3×10^8 cpm per μg) after isolation of the inserts; 9.4- and 5.0-kb bands in the germ-line configuration contain $C_{\beta 1}$ and $C_{\beta 2}$, respectively. Therefore, the intensity of these two bands is brighter when the T-cell receptor gene undergoes rearrangement, while that of the 3.7-, 2.8-, and 0.9-kb bands does not change and serves as control.

receptor genes underwent rearrangement, the 9.4- and 5.0-kb bands containing the $C_{\beta 1}$ and $C_{\beta 2}$ region, respectively, were changed in length. In the heterogenous population, as mentioned above, these fragments were hardly detectable on Southern blots. Therefore, the extent of DNA rearrangement parallels the disappearance of the 9.4- and 5.0-kb bands. On the other hand, the 0.8- and 2.9-kb bands remained unchanged despite rearrangement of the T-cell receptor gene and served as internal controls. In the experiment depicted, a 3.7-kb fragment is seen because of incomplete digestion at the *Hin*dIII restriction site, which splits the 3.7-kb fragment into an 0.8-kb and a 2.9-kb fragment.

Southern analysis (Fig. 2*B*) of the lymph-node T cell-derived DNA obtained from the C3H/HeJ *gld/gld* strain was informative. The 9.4- and 5.0-kb bands in the lanes derived from that strain were barely detectable compared with the lane from the normal C3H/HeJ strain. Despite the fact that restriction enzyme digestion was incomplete, similar intensities of each lane in the 3.7-, 2.9-, and 0.8-kb bands indicates that the restriction enzyme digest of the DNA was equivalent for the C3H/HeJ and C3H/HeJ *gld/gld* DNA.

These experiments with different combinations of restriction enzymes and probes indicate that the germ-line configuration of the T-cell receptor β-subunit gene in the peripheral lymph-node T cells of C3H/HeJ *gld/gld* mice was not maintained at the same degree of rearrangement as seen in the C3H/HeJ strain. In addition, ontogenetic studies showed that, although immunologic abnormalities become apparent at a later stage of life (8), extensive β-subunit gene rearrangements are already evident in young animals (Fig. 2*B*).

Expression of the T-Cell Receptor RNA. When the expression of certain genes in cells is compared among several different samples, it is important to estimate the total amount of mRNA expression of each cell from each sample. We found that lymph node-residing T cells from C3H/HeJ strain and C3H/HeJ *gld/gld* mice contain similar amounts of total and poly(A)$^+$ RNA per cell (data not shown). Although the total and poly(A)$^+$ RNA of thymocytes was slightly less than that of lymph-node cells, the thymus-residing T cells from C3H/HeJ and C3H/HeJ *gld/gld* strains were observed to have identical RNA content.

RNA blot hybridization (Fig. 3*A*) revealed that the level of the β-subunit RNA expression of lymph-node-residing T cells of the C3H/HeJ *gld/gld* strain was much higher than that seen in the normal-counterpart C3H/HeJ strain. On the other hand, the level of β RNA expression was not significantly different between T cells obtained from the thymus of either C3H/HeJ or C3H/HeJ *gld/gld* mice. In normal mice, the level of β RNA expression in the thymus was greater than that in the periphery. However, in the C3H/HeJ *gld/gld* strain, the level of the β RNA expression in the thymus was less than that in the periphery.

RNA blot analysis (Fig. 3*B*) also revealed that the expression of α-subunit RNA in T cells obtained from strain C3H/HeJ *gld/gld* was strikingly higher than that in T cells obtained from C3H/HeJ normal strain in both thymus and lymph node. It was also apparent that C3H/HeJ *gld/gld* T cells in the lymph node expressed the α RNA more than did thymus-residing T cells (Fig. 3*B*). We also examined the level of the γ-subunit RNA in C3H/HeJ and C3H/HeJ *gld/gld* mice. As previously described (27–29), γ RNA expression was much lower than that of the α and β RNAs. On blot analysis (Fig. 3*C*), little difference was observed with respect to the γ RNA found in the lymph node and thymus T cells of C3H/HeJ or C3H/HeJ *gld/gld* strains.

Collectively, the data demonstrates that (*i*) β-subunit RNA expression in C3H/HeJ *gld/gld* thymus-residing T cells is comparable to that seen for C3H/HeJ thymocytes (nevertheless, the "expression" and "rearrangement" of these genes is much more extensive in lymph node-residing T cells of C3H/HeJ *gld/gld* origin than in C3H/HeJ cells); (*ii*) in C3H/HeJ *gld/gld* thymocytes, the amount of expression of the α-subunit RNA is considerably higher than that seen in C3H/HeJ thymus (furthermore, peripheral *gld/gld* lymph nodes also exhibit a level of expression of the α RNA that is even higher than that seen in the thymus); and (*iii*) the levels of expression of the γ RNA are similar in thymus and lymph-node cells of C3H/HeJ and C3H/HeJ *gld/gld* strains.

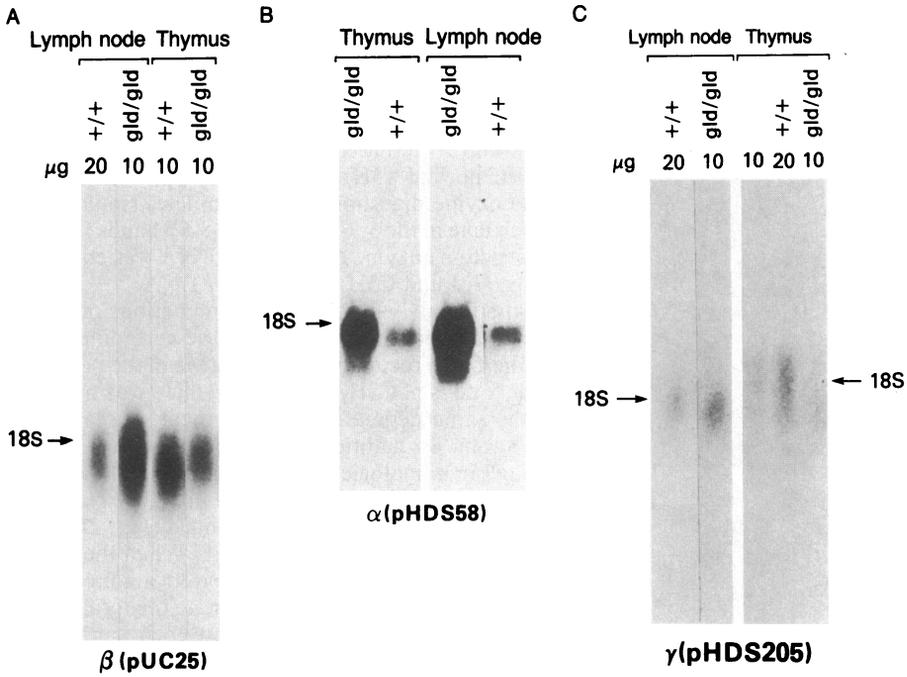

FIG. 3. RNA blot-hybridization analysis of the T-cell receptor α-, β-, and γ-subunit RNAs in T cells from lymph node and thymus of C3H/HeJ +/+ (indicated +/+) and C3H/HeJ *gld/gld* strain (indicated gld/gld). (*A*) Total cellular RNA was extracted from enriched T cells, and 10 or 20 μg of RNA (indicated on the top of each column) was analyzed. Detailed hybridization and washing conditions are described in *Materials and Methods*. Clone pUC25 (Fig. 1) was used as the probe. The blots were exposed for 20 hr. (*B*) RNA blot-hybridization analysis of the T-cell receptor α RNA. The clone pHDS 58 was used as the probe (27). The blots were exposed for 20 hr. (*C*) RNA blot-hybridization analysis of the T-cell receptor γ RNA. The clone pHDS205 was used as the probe (28). The blots were exposed for 80 hr.

DISCUSSION

Rearrangement of the β-Subunit Gene. Rearrangements of immunoglobulin H chain genes are thought to occur in a two-step process. D_H segments are first joined to J_H on both chromosomes, followed by a joining process of V_H to D_H–J_H (30). Occasional joining of D_H–J_H is observed in some T cells as well as in pre-B-cells. However, V_H joining to D_H–J_H is considered to be regulated and indicative of a productive rearrangement. That is, the immunoglobulin genes in which V_H is joined to D_H–J_H are likely to produce protein if the rearrangement has occurred properly. Since the overall process of T-cell receptor gene rearrangement is similar to that operative for immunoglobulin genes, it is likely that V_β joining to D_β–J_β has biological significance. To examine the extent of the β-subunit gene rearrangement, we used two combinations of restriction enzymes and probes. In some cases, our method could not distinguish nonrearranged germline bands from *D–J* joined rearranged bands, because the distance between *D* and *J* segments is small and changes of band length are too slight to detect (see Fig. 2, the *D*-to-*J* distance is very small). However, as discussed above, if we consider a joining of *V* to *D–J* as indicative of a productive rearrangement of the T-cell receptor genes, our Southern blots demonstrate that the extent of "productive" β gene rearrangements in C3H/HeJ *gld/gld* T cells occurs more extensively than in C3H/HeJ peripheral T cells.

There are several possible interpretations of our data. In the first, T-cell receptor genes in C3H/HeJ *gld/gld* strains undergo rearrangement and expression even after emigration from the thymus to the periphery. These continuously rearranging abnormal cells might accumulate in the secondary lymphoid organs. Although peripheral T cells may reenter the thymic environment, this is considered to represent only a small number of cells. Unless *gld* mice also accumulate peripheral T cells in their thymus at a high rate, the observed enhanced level of α-subunit RNA expression in the thymus of the C3H/HeJ *gld/gld* mice does not support the idea that abnormal expression and extensive rearrangement occurs only after T cells emigrate from the thymus to the periphery.

A second possibility relates to the hypothesis that in the healthy mouse, T cells that aberrantly rearrange their T-cell receptor genes are eliminated in the thymus by some process and, therefore, cannot emigrate to the periphery. Although most cells in the thymus express T-cell receptor genes (31), a smaller percentage of human thymocytes have detectable receptor molecules on their surface. It is also known that 99% of thymocytes are eliminated during thymus selection (32). In addition, several out-of-phase T-cell receptor mRNAs are found in the thymus (31), whereas these out-of-phase mRNA species are rarely found in the periphery or in experimentally produced functional T-cell lines and T-cell hybridomas created from peripheral T cells. These observations support the notion that the thymus gland is the site where T cells with nonfunctional genes are selected against. From these data, two independent types of thymus selections have been postulated (31). During the first selection process, most T cells that have nonfunctionally rearranged receptor genes are thought to be eliminated. The second process occurs after receptor molecules are expressed on the cell surface. In this step, T cells are selected for self-reactivity with products of the major histocompatibility complex, and high-affinity interacting cells are somehow eliminated. In the context of this hypothesis, C3H/HeJ *gld/gld* aberrantly rearranged T cells are not deleted because of an ineffective elimination mechanism and emigrate to the periphery. In addition, several other observations also suggest selection failure in the *gld/gld* mouse. First, the bulk of the T cells residing in lymph node in C3H/HeJ *gld/gld* mice are phenotypically immature; their surface phenotypes are similar to immature T cells resident in the normal thymus (8). This suggests that the cells might leave the thymus prior to complete maturation or prior to receiving normal signals that permit further maturation in the periphery. Second, neonatal thymectomy retards the autoimmune process in the *lpr/lpr* mice, which demonstrates an autoimmune disease syndrome similar to that seen in *gld/gld* mice (17). Third, the high level of c-*myb* expression, seen primarily in thymic lymphocytes of normal mice, is also observed in lymphocytes of peripheral lymph nodes of C3H/HeJ *gld/gld* mice (33). These data collectively support the proposition that in the C3H/HeJ *gld/gld* strain, nonselected T cells emigrate to the periphery after escaping elimination in the thymus.

Expression of the α- and β-Subunit RNAs. Recently, several observations suggest that the recombinational joining of immunoglobulin *V*, *D*, and *J* segments is not precisely regulated to conserve the translational reading frame (34). As a consequence, a large portion of precursor B cells contain

nonfunctional genes with out-of-phase joining events or contain improperly located termination codons for the immunoglobulin transcripts. B cells that contain aberrantly rearranged genes appear to be blocked in terms of differentiation (34). In addition, high levels of immunoglobulin mRNA are observed in Abelson murine leukemia virus-transformed pre-B-cells that contain nonfunctional genes in both chromosomes (18). Since expression of the T-cell receptor gene is thought to be regulated by a similar process and common mechanism, T cells containing nonfunctional T-cell receptor genes would be expected to have high levels of T-cell receptor mRNA and would be similarly blocked for further differentiation.

However, we have found that T cells of the C3H/HeJ *gld/gld* strain contain equivalent amounts of total mRNA compared to the C3H/HeJ normal strain. Our data further demonstrate that T cells in the C3H/HeJ *gld/gld* mice express much higher levels of the α-subunit RNA in the lymph node and thymus and of the β-subunit RNA in the lymph node when compared with the C3H/HeJ normal strain. One possible explanation of the high level of T-cell receptor RNA is the following. If the thymus is the site of elimination of T cells, which have nonfunctional genes in the healthy mice, and if this process is imperfect in the *gld* strain thymus, a large portion of T cells with nonfunctional T-cell receptor genes would be allowed to emigrate to the periphery and also would be expected to express high levels of T-cell receptor mRNA. This would be comparable to the pre-B-cell, which contains nonfunctionally rearranged immunoglobulin genes. Another possibility is that intrinsic defects do not regulate rearrangement and expression of the T-cell gene.

Expression of the γ Subunit. Our results did not reveal any significant difference of the γ-subunit gene expression between C3H/HeJ *gld/gld* and C3H/HeJ strains; this observation suggests that γ-subunit gene expression may be regulated by distinct transcriptional or feedback mechanisms and/or may not be involved in proposed thymus selection as mentioned above. Recently, many γ genes containing out-of-phase or termination codons have been found in functional T cells. Therefore, it is unclear whether the γ-subunit gene plays a role in thymus selection or may be relevant to another pathway. For example, γ chain-related selection may occur in only certain subsets of MHC-responsive T cells.

We conclude that abnormalities of α- and β-subunit gene rearrangements and expression are features of the immature populations of T cells that accumulate in the periphery of the *gld* mouse. One mechanism that we speculate may explain this accumulation is that it occurs as a consequence of an imperfect thymus selection process. It remains to be established how and by what means thymus selection occurs and what defects are operative in the *gld* strain.

We are grateful to Dr. Katsuyuki Yui for assistance and Drs. Nobukata Shinohara and Toshiaki Mizuochi for critical reading. This work was supported by grants from the National Cancer Institute (to A.M.M.), the Council for Tobacco Research, the American Cancer Society, and the National Institutes of Health (to M.I.G.).

1. Hood, L., Kronenberg, M. & Hunkapiller, T. (1985) *Cell* **40,** 225–229.
2. Tonegawa, S. (1983) *Nature (London)* **302,** 575–581.
3. Snodgrass, H. R., Kisielow, P., Kiefer, M., Steinmetz, M. & von Boehmer, H. (1985) *Nature (London)* **313,** 592–595.
4. Raulet, D. H., Garman, R. D., Saito, H. & Tonegawa, S. (1985) *Nature (London)* **314,** 103–107.
5. Samelson, L. E., Lindsten, T., Fowlkes, B. J., van den Elsen, P., Terhorst, C., Davis, M. M., Germain, R. N. & Schwartz, R. H. (1985) *Nature (London)* **315,** 765–768.
6. Andrews, B. S., Eisenberg, R. A., Theofilopoulos, A. N., Izui, S., Wilson, C. B., McConahey, P. J., Murphy, E. D., Roths, J. B. & Dixon, F. J. (1978) *J. Exp. Med.* **148,** 1198–1215.
7. Theofilopoulos, A. N. & Dixon, F. J. (1981) *Immunol. Rev.* **55,** 179–216.
8. Roths, J. B., Murphy, E. D. & Eicher, E. N. (1984) *J. Exp. Med.* **159,** 1–20.
9. Mountz, J. D., Mushinski, J. F. & Steinberg, A. D. (1985) *Surv. Immunol. Res.* **4,** 48–64.
10. Altman, A., Theofilopoulos, A. B., Weiner, R., Katza, D. H. & Dixon, F. J. (1981) *J. Exp. Med.* **154,** 791–808.
11. Theofilopoulos, A. N., Shawler, D. L., Katz, D. H. & Dixon, F. J. (1979) *J. Immunol.* **122,** 2319–2327.
12. Fischbach, M. (1984) *J. Immunol.* **133,** 2365–2368.
13. Santoro, T. J., Benjamin, W. R., Oppenheim, J. J. & Steinberg, A. D. (1983) *J. Immunol.* **131,** 265–268.
14. Wofsy, D., Murphy, E. D., Roths, J. B., Dauphinee, M. J., Kipper, S. B. & Talal, N. (1981) *J. Exp. Med* **154,** 1671–1680.
15. Dauphinee, M. J., Kipper, S. B., Wofsy, D. & Talal, N. (1981) *J. Immunol.* **127,** 2483–2487.
16. Santoro, T. J., Luger, T. A., Ravache, E. S., Smolen, J. S., Oppenheim, J. J. & Steinberg, A. D. (1983) *Eur. J. Immunol.* **13,** 601–604.
17. Hang, L., Theofilopoulos, A. N., Balderas, R. S., Francis, S. J. & Dixon, F. J. (1984) *J. Immunol.* **132,** 1809–1813.
18. Kelley, D. E., Wiedemann, L. M., Pittet, A. C., Strauss, S., Nelson, K. J., Davis, J., Van Ness, B. & Perry, R. P. (1985) *Mol. Cell. Biol.* **5,** 1660–1675.
19. Henry, C. (1980) in *Selected Methods in Cellular Immunology*, eds. Mishell, B. B. & Shiigi, S. M. (Freeman, San Francisco), pp. 182–185.
20. Marshak-Rothstein, A., Fink, P., Gridley, T., Raulet, D. H., Bevan, M. J. & Gefter, M. L. (1979) *J. Immunol.* **122,** 2491–2497.
21. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
22. Bier, E., Hashimoto, Y., Greene, M. I. & Maxam, A. M. (1985) *Science* **229,** 528–534.
23. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
24. Chirgwin, J., Przybyla, A., MacDonald, R. & Rutter, W. (1979) *Biochemistry* **18,** 5294–5299.
25. Thomas, P. S. (1979) *Methods Enzymol.* **100,** 255–266.
26. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
27. Saito, H., Kranz, D. M., Takagaki, Y., Hayday, A. C., Eisen, H. N. & Tonegawa, S. (1984) *Nature (London)* **312,** 36–40.
28. Saito, H., Kranz, D. M., Takagaki, Y., Hayday, A. C., Eisen, H. N. & Tonegawa, S. (1984) *Nature (London)* **309,** 757–762.
29. Chien, Y.-h., Becker, D. M., Lindsten, T., Okamura, M., Cohen, D. I. & Davis, M. M. (1984) *Nature (London)* **312,** 31–35.
30. Alt, F. W., Yancopoulos, G. D., Blackwell, T. K., Wood, C., Thomas, E., Boss, M., Coffman, R., Rosenberg, N., Tonegawa, S. & Baltimore, D. (1984) *EMBO J.* **3,** 1209–1219.
31. Kavaler, J., Davis, M. M. & Chien, Y.-h. (1984) *Nature (London)* **310,** 421–423.
32. Scollay, R. G., Butcher, E. C. & Weissman, I. L. (1980) *Eur. J. Immunol.* **10,** 210–217.
33. Mountz, J. D., Steinberg, A. D., Klinman, D. M., Smith, H. R. & Mushinski, J. F. (1984) *Science* **226,** 1087–1089.
34. Hagiya, M., Davis, D. D., Takahashi, T., Okuda, K., Raschke, W. C. & Sakano, H. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 145–149.