gistic in the presence of competent T cells. This property of HBcAg may explain its strong immunogenicity. However, not all antigens of this general type are T cell–independent (for example, HBsAg). It has been suggested that a T-cell–independent molecule must contain a threshold number of appropriately spaced haptens or epitopes necessary for simultaneous B-cell receptor binding (*18*). The two HBV particulate antigens HBcAg and HBsAg may differ in this respect. The nucleocapsid protein of HBV, like that of other animal viruses, possesses protein kinase activity (*19, 20*). The rHBcAg used in these experiments exhibited such activity, and the influence of this property of HBcAg on immunogenicity warrants further study. The extent to which the enzymatic, structural, and immunologic properties described herein for HBcAg are shared with the nucleocapsid antigens of other viruses remains to be determined.

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Characterization of T Cell Receptor Gamma Chain Expression in a Subset of Murine Thymocytes

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While much information exists about the structure and function of the clonally distributed T cell receptor (TCR) αβ heterodimer, little is known about the γ protein, the product of a third rearranging TCR gene. An antiserum to a carboxyl-terminal peptide common to several of the murine gamma chain constant regions and a monoclonal antibody to the murine T3 complex were used to identify products of this TCR gene family in a subpopulation of Lyt2$^-$, L3T4$^-$ thymocytes. This subpopulation does not express TCRα or full-length TCRβ messenger RNA. The gamma chain is a 35-kilodalton (kD) protein that is disulfide-bonded to a 45-kD partner and is associated with the T3 complex. Analysis of the glycosylation pattern of this thymic gamma chain revealed that the major variable region gamma (Vγ) gene transcribed in activated peripheral T cells is absent from this subpopulation. The cells that bear this second T cell receptor may therefore represent a distinct lineage differentiating within the thymus.

ANTIGEN-SPECIFIC, MHC-RESTRICTED recognition by mature T cells appears to be mediated by the clonotypic αβ heterodimeric receptor (*1*). These proteins are noncovalently associated with the multicomponent T3 complex, which appears to be responsible for transduction of receptor-mediated activation signals (*2*). A search for the genes encoding the α and β proteins revealed a third T cell receptor (TCR)–like gene, termed γ, which contains variable (V), joining (J), and constant (C) regions that rearrange in cells of the T lineage (*3*). In contrast to the TCRα and TCRβ genes, there appears to be a much smaller number of Vγ elements (see below) (*4*), implying that TCRγ can make only a limited contribution to the diversity of any receptor involving this chain. A clue to the possible function of the TCRγ product came from the finding that γ messenger RNA (mRNA) is expressed at high levels in early murine fetal thymocytes and analogous Lyt2$^-$, L3T4$^-$ cells from adult thymus (*5*). Since these populations contain precursors to mature T cells (*6*), the γ protein has been postulated to play a role in T cell development. In order to study TCRγ expression at the protein level, we have raised an antiserum to a synthetic peptide corresponding to the seven carboxyl-terminal amino acids of the murine γ chain; the peptide sequence was deduced from the complementary DNA (cDNA) sequence (*3*). Using this antiserum and a monoclonal hamster antibody to the murine T3 complex (termed 145-2C11), we show here that a subset of adult Lyt2$^-$, L3T4$^-$ thymocytes express γ chain on their surface in association with the T3 complex. A similar molecule has been identified in certain T cell populations of human peripheral blood (*7*). The murine γ chain is disulfide-bonded to a second 45-kD protein that is neither the α nor the β chain. All of the detectable γ chain on the surface of these cells is 35 kD and reduces to the predicted core size of 32 kD after treatment with endoglycosidase. This analysis demonstrates that there is no expression at the protein level of the $V_{\gamma 1.2}$-$C\gamma_2$ combination that is the major γ mRNA expressed outside the thymus. These T3$^+$, Lyt2$^-$, L3T4$^-$ cells may therefore represent a distinct lineage of cells within the thymus.

The murine γ gene family has been extensively characterized at the DNA level (*4, 8–10*). A schematic diagram of the genomic structure and probable rearrangement patterns is shown in Fig. 1. There are four known J-C elements (J_1-C_1, J_2-C_2, J_3-C_3, and J_4-C_4) and four known V region families (V_1, V_2, V_3, and V_4); each has one member, except for V_1, which has three members ($V_{1.1}$, $V_{1.2}$, and $V_{1.3}$). Furthermore, V_2, V_3, and V_4 each rearrange to J_1-C_1; $V_{1.2}$ rearranges to J_2-C_2; $V_{1.1}$ rearranges

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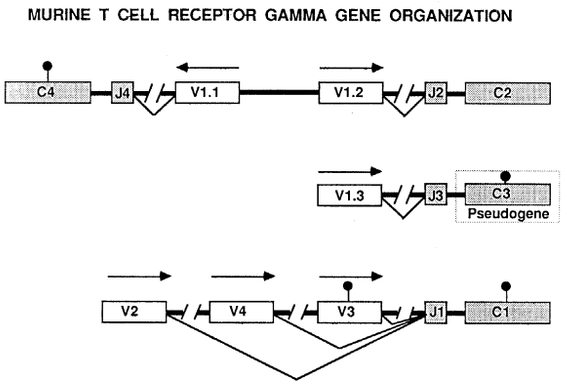

Fig. 1. Schematic diagram of murine T cell receptor gamma genomic organization and rearrangements. The proposed genomic organization of the γ gene family outlined above is a compilation of the studies of Hayday *et al.* (*8*), Garman *et al.* (*4*), Helig and Tonegawa (*9*), and Iwamoto *et al.* (*10*). The nomenclature used is that of Garman *et al.* (*4*), although an alternative nomenclature has recently been proposed (*9*). Lines that connect the 3′ end of V regions to the 5′ end of J regions represent potential rearrangements, all of which have been documented (*4, 8–10*) except for $V_{1.3}$-J_3-C_3. Direction of transcription is denoted with arrows above the V regions. Potential sites for *N*-linked glycosylation are denoted with lollipops.

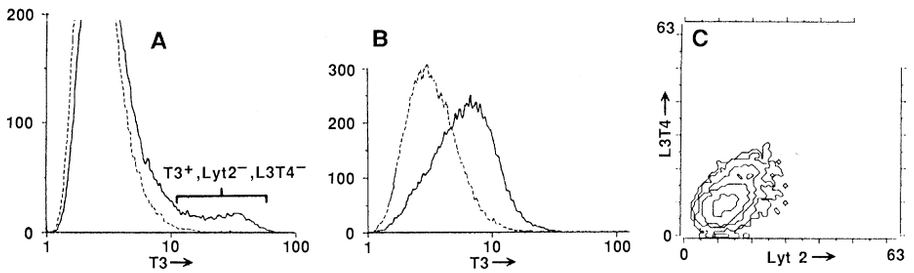

Fig. 2. Surface T3 expression of freshly isolated and cultured Lyt2$^-$, L3T4$^-$ thymocytes. (A) Lyt2$^-$, L3T4$^-$ cells, isolated from fresh adult B6 mouse thymus by cytotoxic elimination with monoclonal antibodies to Lyt2, L3T4, and Ly1.2, were stained with the monoclonal antibody to T3, 145-2C11, followed by fluorescein isothiocyanate (FITC)–labeled mouse antibody to hamster immunoglobulin (Ig) (——) or with the mouse antibody to hamster Ig (------). (B) Lyt2$^-$, L3T4$^-$ cells cultured 4 days in recombinant IL-1, recombinant IL-2, WEHI-3 conditioned medium and supernatant from Con A–stimulated rat spleen cells were stained as above with anti-T3 followed by FITC-labeled mouse antibody to hamster Ig (——) or with FITC-labeled mouse antibody to hamster Ig alone (------). (C) Two-color analysis of Lyt2$^-$, L3T4$^-$ cells cultured 4 days as above. Lyt2 is represented on the *x*-axis and L3T4 is represented on the *y*-axis. Cells were stained with FITC-labeled antibody to Lyt2 and biotinylated MT4 (anti-L3T4), and then by Texas Red avidin. The Lyt2$^-$, L3T4$^-$ thymocytes were isolated (*6*) by sequential cytotoxic elimination with a mouse antibody to Ly1.2, GK1.5 (rat antibody to L3T4) facilitated with MAR 18.5 (mouse antibody to rat kappa chain) and 19/178 (mouse antibody to Lyt2.2). Four-day cultured Lyt2$^-$, L3T4$^-$ cells were grown in Dulbecco's medium with 10 percent fetal calf serum, recombinant IL-1 (20 U/ml), recombinant IL-2 (100 U/ml), the supernatant (10 percent) from Con A–stimulated rat spleen cells (from which lectin was removed) and WEHI-3 conditioned medium (20 percent). Cell sorter analysis was performed on live cells with a FACS II (Becton Dickinson).

to J_4-C_4; and $V_{1.3}$ is linked to C_3 which is a pseudogene. At the RNA level, only the V_1 family appears to be expressed significantly in activated mature T cell clones and peripheral T cells (*4*), whereas $V_{1.2}$, V_2, V_3, and V_4 are all expressed in developing thymocytes (*4*). However, $V_{1.1}$-C_4 is not expressed at detectable levels in thymocytes (*9*). To investigate γ expression at the protein level, we generated, by previously described methods (*11*), a rabbit antiserum against the heptapeptide (CGNEKKS) conjugated to keyhole limpet hemocyanin (*12*) that corresponds to the carboxyl terminus of C_1, C_2, and C_3. This antiserum (anti-γ) has the potential to recognize the majority of products of the known γ genes.

The monoclonal antibody to T3 (anti-T3) used in our study was made in Armenian hamsters and is specific for the 25-kD epsilon component of the murine T3 complex. Its recognition and activation properties have been described (*13*).

Because γ mRNA levels are high in immature thymocytes, we initially studied these subpopulations. About 3 to 4 percent of adult thymocytes express low levels of Ly1, but no Lyt2 or L3T4 (*6*). This subpopulation resembles fetal thymocytes from day 15 to 16 of gestation in that both are Lyt2$^-$, L3T4$^-$ (*14*), have IL-2 (interleukin 2) receptors (*15*), and express high levels of TCRβ and TCRγ mRNA, but little, if any, TCRα mRNA (*5*).

Immunofluorescent staining of Lyt2$^-$, L3T4$^-$ cells with the anti-T3 revealed that, as with human thymocytes (*16*), a portion (~5 percent) express high levels of T3 (Fig. 2A). In mature phenotype T cells, there is a requirement for coordinate expression of T3 with an αβ heterodimer (*2*). Because of the absence of TCRα mRNA and the elevated levels of TCRγ mRNA in the Lyt2$^-$, L3T4$^-$ subpopulation (*5*), we sought to determine whether the T3 molecules expressed on these cells were associated with a TCRγ protein. This association was demonstrated when cells surface-labeled with ^{125}I were lysed in the mild detergent digitonin, which has been shown to keep the TCRαβ/T3 complex intact (*17*).

Immunoprecipitation of surface-labeled, unfractionated adult mouse thymocytes with the anti-γ did not reveal any evidence of gamma protein (Fig. 3A). Immunoprecipitation with the anti-T3 showed the expected αβ components in addition to the 21-kD and 25-kD T3 components (Fig. 3A). However, both the anti-γ and anti-T3 immunoprecipitated a 35-kD protein from freshly isolated Lyt2$^-$, L3T4$^-$ cells (Fig. 3B). By contrast, the size of the α and β chains is 38 kD to 43 kD. When excess γ peptide was present during the anti-γ immunoprecipitation, the 35-kD band was eliminated (first lane), implying that this protein is specifically immunoprecipitated by the anti-γ (second lane). Immunoprecipitation with anti-T3 revealed an additional 45-kD protein (third lane) not easily seen with the anti-γ because of the effect of the immunoglobulin front, which caused background bands to merge in this region. Both the anti-γ and anti-T3 immunoprecipitated an 80-kD protein under nonreducing conditions (Fig. 3C), indicating that the 35-kD and 45-kD proteins exist as a disulfide-linked heterodimer. Therefore, at least some of the γ mRNA present in this cell population appears to be translated into an expressed membrane protein. Only the 21-kD T3 component was visualized in anti-T3 immunoprecipitations of Lyt2$^-$, L3T4$^-$ cells. The difference between the T3 molecules in these cells may be due to structural differences between αβ-associated T3 and γ-associated T3.

Further biochemical characterization of the γ receptor complex was facilitated by a procedure that enabled us to selectively grow out T3$^+$, Lyt2$^-$, L3T4$^-$ thymocytes. When these cells were cultured in a combination of IL-1, IL-2, rat Con A (concanavalin A) supernatant, and WEHI-3–conditioned medium (as a source of IL-3), T3$^+$ cells proliferated. After 4 days of culture, most of the live cells were T3$^+$ while retaining the Lyt2$^-$, L3T4$^-$ phenotype (Fig. 2, B and C). The pattern seen on one-dimensional reducing SDS-PAGE (polyacrylamide gel electrophoresis) gels of anti-γ and anti-T3 immunoprecipitates of the 4-day cultured cells is virtually identical to freshly isolated Lyt2$^-$, L3T4$^-$ cells (Fig. 3D), suggesting that, at the biochemical level, they are representative of the in vivo T3$^+$, Lyt2$^-$, L3T4$^-$

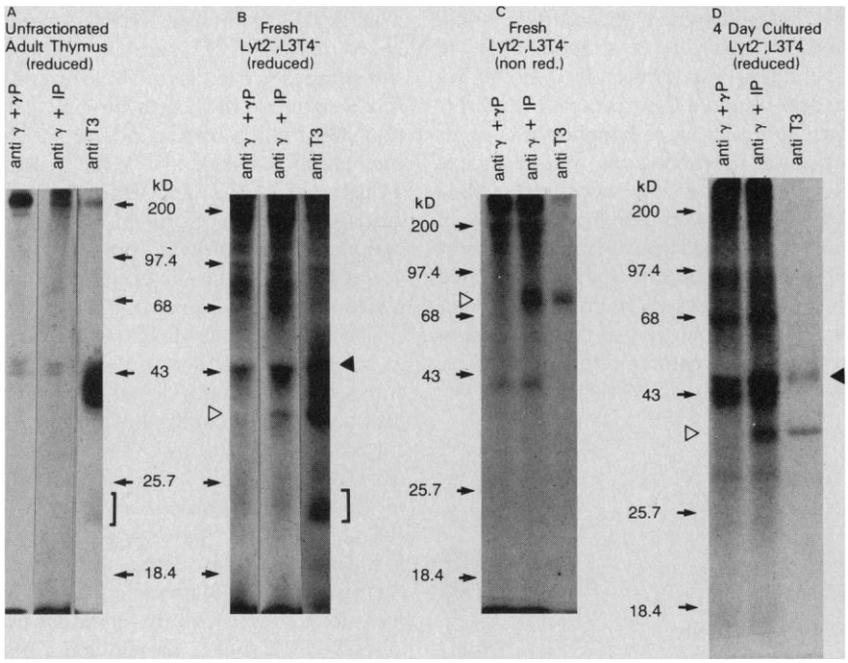

Fig. 3. Immunoprecipitation of unfractionated thymocytes and freshly isolated Lyt2$^-$, L3T4$^-$ thymocytes with anti-γ and anti-T3 antibody. (A) Unfractionated thymocytes, (B and C) fresh Lyt2$^-$, L3T4$^-$ cells, and (D) 4-day cultured (majority T3$^+$) Lyt2$^-$, L3T4$^-$ cells were ^{125}I surface–labeled, lysed with NP40 (for anti-γ) or digitonin (for anti-T3), and tested by immunoprecipitation with the anti-γ plus γpeptide (γP) (lane 1 each panel), anti-γ plus irrelevant peptide (IP), or the anti-T3. All samples were run on SDS-PAGE gels under reducing conditions except for C which was run under nonreducing conditions. As described in Fig. 1, Lyt2$^-$, L3T4$^-$ cells were isolated fresh or cultured for 4 days. Cells (1×10^7 to 5×10^7) were ^{125}I-labeled by a lactoperoxidase procedure (*10*). Cell viability was >95 percent after ^{125}I labeling. Cells were then lysed in 0.5 percent NP40 or 1 percent digitonin containing phenylmethylsulfonyl fluoride, leupeptin, pepstatin, and chymostatin. Samples were then cleared with rabbit antiserum to chicken Ig. Immunoprecipitates were collected with protein A agarose beads, washed four times, dissociated by boiling in 2 percent SDS sample buffer, and run under reducing (A, B, and D) or nonreducing (C) conditions on 10 percent SDS-PAGE gels at 6 mA per gel for 12 to 15 hours (*10*). For peptide inhibition studies, 100 μg of the immunizing γ peptide was added during the immunoprecipitation. Gels were dried, and labeled proteins were visualized with the use of Kodak XAR-5 autoradiographic film at −70°C with enhancing screens.

population. Diagonal gels (first dimension under nonreducing conditions, second dimension under reducing conditions) from anti-γ and anti-T3 immunoprecipitations of these 4-day cultured cells (Fig. 4) show a 35-kD and a 45-kD protein below the diagonal in both cases. These two spots were completely absent when excess γ peptide was added to the anti-γ immunoprecipitation. This experiment proves that the receptor on these cells is a disulfide-linked heterodimer.

In order to prove the association of this heterodimer with T3, sequential immunoprecipitations were performed. "Preclearance" with anti-T3 completely removed the 35-kD protein specifically precipitated by the anti-γ (Fig. 5). Preclearance with anti-γ removes most but not all of the material precipitated by anti-T3. The failure to completely preclear with the anti-γ could be due to inefficiency of the serum (by incomplete immunoprecipitation) or possibly to the association of the T3 complex with other γ proteins not recognized by this antiserum.

In order to determine which of the two T3-associated proteins reacts with the anti-γ, we subjected digitonin lysates (*17*) to immunoprecipitation with anti-T3. After elution of the precipitate with SDS, the disulfide bond between the two chains of the heterodimer was broken by reduction with dithiothreitol, and subsequent alkylation with iodoacetamide. The noncovalently linked chains of the T3-associated heterodimer were then immunoprecipitated with the anti-γ and run on an SDS-PAGE gel. Under these conditions, only the 35-kD protein is seen (Fig. 6A), indicating that this molecule is the γ chain. This experiment also confirms that the γ protein is T3-associated.

To gain information on Vγ expression in these cells, we used the enzyme N-glycosidase F, which removes N-linked carbohydrate groups. This analysis is informative for the murine γ proteins because the $V_{\gamma 1.2}$-$C_{\gamma 2}$ chain has no N-linked carbohydrate acceptor sites (*3*). Both the γ chain and its 45-kD partner are sensitive to N-glycosidase F (Fig. 6B). It appeared that the γ chain reduced to the predicted core size of 32 kD and its partner reduced to roughly 36 kD. To confirm that all of the γ protein is sensitive to N-glycosidase F, anti-γ immunoprecipitates were treated with N-glycosidase F, reduced, alkylated, and then reprecipitated with the anti-γ. Under these conditions, all the 35-kD material reduced to 32 kD and no 35-kD material is present in the sample treated with N-glycosidase F (Fig. 6C). Taken together, these results indicate

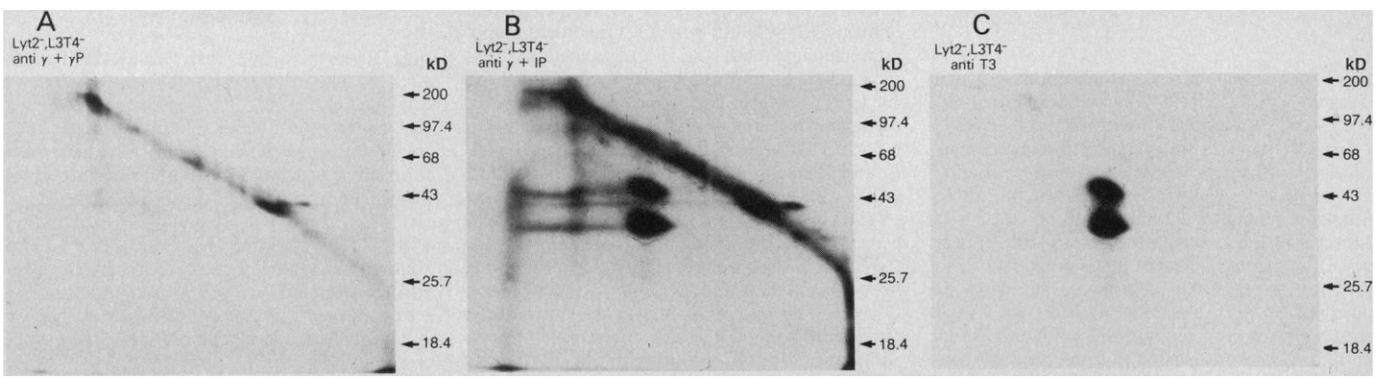

Fig. 4. Diagonal gels of anti-γ and anti-T3 immunoprecipitates of 4-day cultured Lyt2$^-$, L3T4$^-$ cells. Four-day cultured cells were surface-labeled with ^{125}I and lysates were prepared as in Fig. 3. Immunoprecipitates with (A) anti-γ plus γP, (B) anti-γ plus IP, and (C) anti-T3, were run without reduction in the first dimension and then under reducing conditions in the second dimension. As in Fig. 1, Lyt2$^-$, L3T4$^-$ thymocytes were isolated and cultured. Cells were labeled and immunoprecipitated as in Fig. 3. Samples were then eluted in nonreducing sample buffer and run for 12 hours in a 7.5 percent SDS-PAGE tube gel at 0.25 mA per tube. Tube gels were incubated in reducing sample buffer for 2 hours at room temperature, layered on top of a 10 percent SDS-PAGE slab gel and run for 12 hours at 6 mA/gel. The γ peptide inhibition was performed as in Fig. 3.

that all of the 35-kD γ chain is N-glycosylated. The important conclusion from this analysis is that, in this thymic subpopulation, there is no detectable expression of the $V_{\gamma 1.2}$-$C_{\gamma 2}$ gene; which should have a native molecular mass of 32 kD and be N-glycosidase resistant.

The expression of α, β, and γ mRNA was assessed in the $T3^+$, 4-day cultured cells by Northern blotting. Full-length γ mRNA is present, whereas no α mRNA is detected (Fig. 7). These results are similar to what is seen in freshly isolated $Lyt2^-$, $L3T4^-$ cells (*5*). However, in sharp contrast to total $Lyt2^-$, $L3T4^-$ cells, which possess abundant 1.3-kb TCRβ mRNA, the 4-day cultured $Lyt2^-$, $L3T4^-$ cells have some 1.0-kb but no detectable full-length 1.3-kb β mRNA. This result makes it extremely unlikely that the 45-kD partner molecule is the β chain. We therefore find no evidence for a γβ or αβ heterodimer in these cells. If the 4-day cultured $Lyt2^-$, $L3T4^-$ cells are representative of the in vivo $T3^+$, $Lyt2^-$, $L3T4^-$, subset, these results imply that all of the mature TCRβ mRNA seen in freshly isolated $Lyt2^-$, $L3T4^-$ cells resides within the $T3^-$ subset.

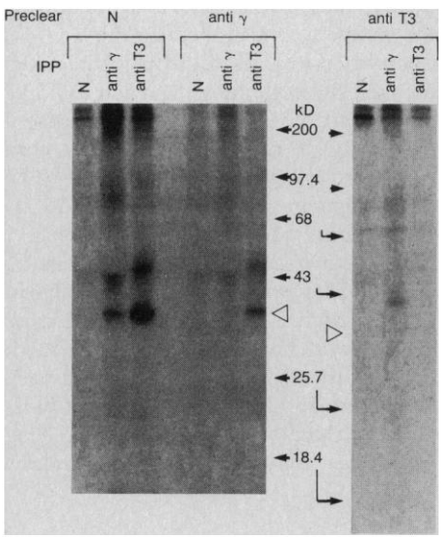

Fig. 5. Sequential immunoprecipitations of 4-day cultured $Lyt2^-$, $L3T4^-$ thymocytes. Digitonin lysates of ^{125}I-labeled 4-day cultured $Lyt2^-$, $L3T4^-$ thymocytes were first immunoprecipitated with normal rabbit antiserum, anti-γ, or anti-T3. Each of these cleared samples was split three ways and immunoprecipitated with normal rabbit antiserum (lane 1, each panel), anti-γ (lane 2, each panel), or anti-T3 (lane 3, each panel). The position of the 35-kD γ protein is shown with open arrows. All the material in the 40- to 45-kD region of anti-γ precipitations represents background. Cells were isolated, cultured, and iodinated as described in Figs. 2 and 3. For the preclearance, lysates were incubated for 12 hours at 4°C with protein A agarose and 20 times more antiserum or antibody than was used for immunoprecipitation. After centrifugation, the supernatant was split into three equal parts and immunoprecipitated as described in Fig. 3.

We have identified the murine T cell receptor γ chain in a subpopulation of $Lyt2^-$, $L3T4^-$ thymocytes. In so doing, we have demonstrated the γ protein in a freshly isolated population of lymphocytes. In its biochemical properties, the murine thymic TCRγ is similar to the recently described human TCRγ (*7*) on peripheral cell lines of certain immunodeficiency patients in that it is present as a heterodimer and is associated with T3. In both cases, there is a difference between the gel pattern of the T3 components of the γ receptor and the αβ receptor. However, as opposed to the described human TCRγ, the murine TCRγ is disulfide-linked to its 45-kD partner. This is not surprising, since unlike the human, the murine γ sequence has a fifth cysteine at position 234 (which corresponds to the interchain disulfide linkage site on the TCRα and TCRβ chains) (*4*). The larger size of the described human γ chain (55 kD) could be due to a larger protein core size (40 kD versus 32 kD) and more potential N-linked glycosylation sites (5 versus 1 to 2).

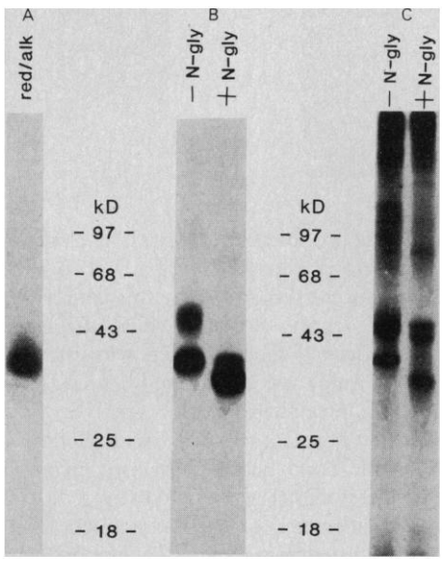

Fig. 6. Reduction-alkylation and *N*-glycosidase F treatment of 4-day cultured $Lyt2^-$, $L3T4^-$ thymocytes. (A) A digitonin lysate of 4-day cultured $Lyt2^-$, $L3T4^-$ cells surface-labeled with ^{125}I was immunoprecipitated with anti-T3, eluted with SDS, reduced with dithiothreitol, alkylated with iodoacetamide, and immunoprecipitated with anti-γ. The immunoprecipitate was run on a 10 percent reducing SDS-PAGE gel. (B) Cells were immunoprecipitated with anti-T3 and eluted as above. Half the sample was treated with N-glycosidase F (lane 2) and half was not (lane 1). After treatment, samples were run on a 10 percent reducing SDS-PAGE gel. (C) Cells were immunoprecipitated with anti-γ and treated with N-glycosidase as in (B); they were then reduced and alkylated as in (A). They were immunoprecipitated again with anti-γ and run on a 10 percent reducing SDS-PAGE gel. All the bands in the 40- to 45-kD region represented background, which was not inhibited by γ-peptide. Cells were isolated and cultured as in Fig. 2. One-dimensional 10 percent SDS-PAGE was performed as in Fig. 3. (A) Anti-T3 immunoprecipitates were eluted from protein A agarose with 0.5 percent SDS in 0.1*M* sodium phosphate buffer, *p*H 8.6; reduced (after bubbling N_2 through the sample) by the addition of 20 m*M* dithiothreitol for 30 minutes at 45°C. Alkylation was performed with 40 m*M* iodoacetamide for 20 minutes at 20°C; a 1/15 volume of 10 percent NP40 was then added, followed by immunoprecipitation with the anti-γ. For (B) and (C), N-glycosidase F (N-Glyconase, TM, Genzyme, Boston, MA) treatment was performed as previously described (*24*).

The identity of the 45-kD protein remains to be elucidated. It does not react with our anti-γ, but we cannot rule out that it is also a member of the γ family. Because the 4-day cultured $T3^+$, $Lyt2^-$, $L3T4^-$ population has no TCRα or full-length TCRβ mRNA, the 45-kD protein cannot be either of these chains. The possibility that the 45-kD protein is a class I molecule has been raised by the report of a T3-associated heterodimer on a cloned human Sezary tumor consisting of a 38-kD "Tβ-like" protein and a 45-kD class I molecule (*18*). However, preclearance of $Lyt2^-$, $L3T4^-$ cell lysates with antiserum to the class I framework (*19*) did not eliminate the γ chain (*20*). Our data, together with the recent report of a T3-associated heterodimer other than αβ in a clone of human $T4^-T8^-$ thymocytes (*21*), definitely demonstrates that developing thymocytes do not require complete α and β chains to express surface T3.

Since all of the cell surface γ chain in the

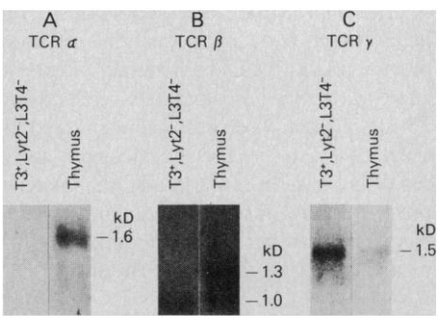

Fig. 7. Northern blot analysis of 4-day cultured $Lyt2^-$, $L3T4^-$ thymocytes. RNA isolated from 4-day cultures of $Lyt2^-$, $L3T4^-$ thymocytes (lane 1 each panel) or unfractionated thymus (lane 2 each panel) was electrophoresed on a 1 percent agarose-formaldehyde gel, blotted onto nitrocellulose, and probed with ^{32}P-labeled probes for (A) TCRα, (B) TCRβ, and (C) TCRγ. RNA was isolated with 4*M* guanidine-HCl, ultracentrifugation through CsCl, phenol-chloroform extraction, and ethanol precipitation. (4 μg of RNA per lane) was separated on a 1 percent agarose formaldehyde gel and blotted onto nitrocellulose (*10*). DNA probes corresponding to the constant regions of TCRα, -β, and -γ ($C_\gamma 2$) were labeled with ^{32}P with the use of nick-translation (*25*), and hybridizations were carried out at 42°C for 12 hours. Blots were washed to a final stringency of 0.1× SSC (standard saline citrate) at 60°C. Blots were dried and exposed to Kodak XAR-5 autoradiographic film at −70°C with enhancing screens.

4-day cultured cells appears to be T3-associated, it is likely that, within the fresh Lyt2$^-$, L3T4$^-$ population, this receptor is predominantly expressed on the T3$^+$ subset of Lyt2$^-$, L3T4$^-$ thymocytes. Are these T3$^+$ cells the precursors to mature T cells bearing αβ and, if so, does the γ chain have a direct role in the thymic influence on receptor repertoire development? To postulate such a role, one would need to invoke a two-receptor model (*21, 22*) in which the receptor containing the γ-chain was positively selected in the thymus and then reexpressed on mature T cells as a second receptor (in addition to αβ) with self-MHC specificity. The N-glycosidase experiments are at variance with such a model; the fact that all of the thymically expressed γ chain is N-glycosylated indicates a total absence of the $V_{1.2}$-C_2 combination, which is the major γ mRNA expressed in mature clones and peripheral T cells. Furthermore, neither the anti-T3, nor the anti-γ precipitated any γ protein from the surface of three activated mature cytotoxic lymphocyte (CTL) clones that transcribe full-length γ mRNA (*20*).

Because $V_{\gamma 2}$-$C_{\gamma 1}$ rearrangements are seen in mature T cells, our data do not completely rule out the precursor nature of T3$^+$, Lyt2$^-$, L3T4$^-$ thymocytes. It is possible that all of the thymic 35-kD γ chain is derived from the $V_{\gamma 2}$-$C_{\gamma 1}$ rearrangement, which is subsequently not transcribed in mature T cells, or that $V_{\gamma 3}$-$C_{\gamma 1}$ and $V_{\gamma 4}$-$C_{\gamma 1}$ rearrangements are expressed in thymocyte precursors and then deleted by a V-gene replacement with $V_{\gamma 2}$ (*4, 23*). However, we favor the more likely possibility that these cells represent a distinct thymus-dependent lineage. This subpopulation may regulate the differentiation of T3$^-$, Lyt2$^-$, L3T4$^-$ precursors to mature αβ receptor-bearing cells. Alternatively, they may be exported and exert their effector function extrathymically. It is now important to determine the functional characteristics of this TCR γ-bearing thymocyte subpopulation.

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Determination of Junction Avidity of Cytolytic T Cell and Target Cell

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A direct measurement of the avidity of the junction between a cytotoxic T lymphocyte and its target cell was achieved by using a biophysical approach. A micromanipulation technique was used to determine the force required to separate a cytotoxic T cell (human clone F1, with specificity for HLA-DRw6) from its specific target cell (JY: HLA-A2, -B7, -DR4, w6) prior to delivery of the lethal hit. The force required to separate the F1-JY pair is 1.5 × 10^4 dynes per square centimeter. This junction avidity for F1-JY pairs is 6 to 13 times greater than that for F1-F1 and JY-JY pairs; the F1-JY conjugate requires a stronger separating force and is more easily rejoined than the homologous cell pairs. This study provides an estimate of the avidity of cytotoxic T cells for their target cells and insights into the biophysical correlates of the molecular complexes formed in the interaction of cytotoxic T cells and their targets during the cytotoxic process.

THE MOLECULAR MECHANISM OF cell-mediated cytolysis has been the subject of several investigations (*1–5*). Long-term allospecific human cytolytic T lymphocyte (CTL) lines have been developed and characterized and are useful for the study of the mechanism of killing at the molecular and cellular level (*6–9*). The interdigitation of the plasma membranes at the contact region between the CTL and its target cell (TC) (*10, 11*), and the changes in the centrioles, the Golgi system (*12*), and the microfilamental-microtubular networks (*13, 14*) have been examined by electron microscopy and immunofluorescence microscopy.

Specific antigen and receptor interaction between a CTL and its TC leads to a reorientation of actin microfilaments and microtubules; this interaction can be inhibited by monoclonal antibodies (*15–18*). The first step of cell-mediated cytolysis is the conjugation of a CTL with its specific TC, and this CTL-TC conjugation could be a commitment step for cytolysis. Our aim was to use the micromanipulation technique with micropipettes to perform quantitative measurements under direct microscopic observation on single CTL-TC pairs, in order to deduce the interaction forces between

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