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Immunology

Characterization of immature thymocyte lines derived from T-cell receptor or recombination activating gene 1 and p53 double mutant mice

(immature T cells)

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ABSTRACT **The T-cell receptor (TCR) β chain is instrumental in the progression of thymocyte differentiation from the $CD4^-CD8^-$ to the $CD4^+CD8^+$ stage. This differentiation step may involve cell surface expression of novel CD3–TCR complexes. To facilitate biochemical characterization of these complexes, we established cell lines from thymic lymphomas originating from mice carrying a mutation in the p53 gene on the one hand and a mutation in TCR-α, TCR-β, or the recombination activating gene 1 (RAG-1) on the other hand. The cell lines were $CD4^+CD8^+$ and appeared to be monoclonal. A cell line derived from a RAG-1 × p53 double mutant thymic lymphoma expressed low levels of CD3-ε, -γ, and -δ on the surface. TCR-α × p53 double mutant cell lines were found to express complexes consisting of TCR-β chains associated with CD3-ε, -γ, and -δ chains and CD3-ζζ dimers. These cell lines will be useful tools to study the molecular structure and signal transducing properties of partial CD3–TCR complexes expressed on the surface of immature thymocytes.**

T-cell development occurs mainly in the thymus, from fetal liver- or bone marrow-derived stem cells that require contact with the thymic stroma for further differentiation. Stages in thymocyte development are conventionally defined by surface expression of the coreceptor molecules CD4 and CD8 (see ref. 1 for a recent review). In the mainstream of αβ thymocyte development, cells progress from the $CD4^-CD8^-$ (double-negative, DN) stage to the $CD4^+CD8^+$ (double-positive, DP) stage, and further to the $CD4^+CD8^-$ or $CD4^-CD8^+$ (single-positive, SP) stage. SP T cells leave the thymus to seed peripheral lymphoid organs.

The T-cell receptor (TCR) on αβ T cells is composed of a clonally variable heterodimer of disulfide-linked TCR-α and -β chains, which are the products of somatically rearranged genes, and of nonpolymorphic CD3-γ, -δ, -ε, and -ζ/η proteins, which are noncovalently associated with each other and with the TCR chains (2, 3). Successful TCR rearrangement or expression determines maturation of thymocytes (1). The first checkpoint is at the DN stage, more precisely at the $CD44^-CD25^+$ DN stage, where production of a successfully rearranged TCR-β chain allows progression to the DP stage and leads to several rounds of cell division (4, 5). The second checkpoint is the DP stage, where production of a successfully rearranged TCR-α chain leads to surface expression of complete TCR–CD3 complexes, which mediate positive and negative selection (1, 4).

The generation of mice with targeted mutations in TCR-α, TCR-β, or the recombination activating gene 1 (RAG-1) has previously enabled us to show that a rearranged TCR-β chain is both necessary and sufficient for differentiation beyond the first checkpoint (6–8). Consequently, TCR-α mutant mice, in which TCR-β gene rearrangement occurs normally, contain a wild-type number of thymocytes, most of which are DP (7). Low surface expression of TCR-β and CD3-ε was detected by flow cytometry on a fraction of thymocytes of our TCR-α mutant mice (7), and this observation was later confirmed (9) in another strain of TCR-α mutant mice (10). The low expression level and the presumed heterogeneity of the TCR-β chains render it difficult to characterize biochemically the immature TCR complexes expressed on the surface of TCR-α mutant thymocytes. This prompted us to attempt to derive clonal cell lines from these thymocytes. Since mice homozygous for a mutation in the tumor suppressor gene p53 develop thymic lymphomas at high frequency (11, 12), we tried to achieve this goal by crossing our mutant mice with p53 mutant mice. To study differentiation stages prior to the expression of TCR-β chains, we crossed our TCR-β or RAG-1 mutant mice with p53 mutant mice. To study stages at which TCR-β is expressed but TCR-α is not yet, we crossed our TCR-α mutant mice with p53 mutant mice. Here, we report the establishment of cell lines from thymic lymphomas generated in TCR × p53 or RAG-1 × p53 double mutant mice. We present an initial characterization of the immature CD3–TCR complexes by flow cytometry, Western blotting, and surface iodination.

MATERIALS AND METHODS

Mice. The generation of TCR-α, TCR-β, RAG-1, or p53 mutant mice by gene targeting in embryonic stem cells has been reported previously (6–8, 12). The mice were in a mixed 129/Sv × C57BL/6 background and were maintained as described (13). These mutant mice are available from The Jackson Laboratory.

Establishment of Cell Lines. Parts of the thymic lymphoma were minced with forceps in a tissue culture dish. The cells were transferred to a T25 flask (Corning) in 10 ml of medium and incubated at 37°C in a 5% CO_2 atmosphere. Two to 3 days later, the cells were diluted 10-fold in the same flask, and subsequently they were passaged every 2–4 days at 10- or 20-fold dilutions into a new T25 flask. Lymphomic cells both grew in suspension and adhered weakly to the dish. A minority of the thymic lymphomas either lost surface expression of TCR-β or stopped growing altogether within 2 weeks. The

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Abbreviations: DN, double-negative; DP, double-positive; FITC, fluorescein isothiocyanate; mAb, monoclonal antibody; PE, phycoerythrin; RAG-1, recombination activating gene 1; TCR, T-cell receptor.

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other lymphomas continued vigorous growth in culture, without crisis. These cell lines were maintained continuously for several months, without substantial loss of surface markers, and could be repeatedly frozen and thawed. Some cell lines contained a minor contamination of cells with an epithelial morphology, which could be diluted out by a gentle resuspension of the cells when passaging the line. For biochemical experiments, cells were grown in T75 or T175 flasks (Corning) at a maximal density of 4×10^6 cells per ml. The culture medium was Dulbecco's modified Eagle's medium (GIBCO), supplemented with 10% heat-inactivated fetal calf serum (Hyclone), 1% L-glutamine stock solution (GIBCO), 1% penicillin–streptomycin stock solution (GIBCO), and 1% 2-mercaptoethanol stock solution (100 μl in 140 ml of phosphate-buffered saline). EL-4 was purchased from the American Type Culture Collection and 3DO-58.4 (14) was a gift from Pablo Pereira (Massachusetts Institute of Technology).

Flow Cytometry. Analysis of surface markers was carried out as described in detail previously (7, 8), except that blocking with serum was found not to be necessary.

Antibodies. H57-597, a monoclonal antibody (mAb) reactive with all TCR-β chains, and 145-2C11, a mAb against CD3-ε, were purchased in purified, fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-labeled form from PharMingen. A panel of biotinylated TCR-Vβ-specific antibodies (V, variable region) was kindly provided by Osami Kanagawa (Washington University, St. Louis). N39 is an anti-peptide serum specific for CD3-ζ (15). Affinity-purified anti-CD3-ε antibody, raised against a peptide comprising amino acids 156–168 of human and mouse CD3-ε, was purchased from Dako.

Surface Iodination. Cells were washed in phosphate-buffered saline and labeled with carrier-free ^{125}I (New England Nuclear) as previously described (16).

Immunoprecipitation. Cells were lysed in a buffer containing 1% Brij-35 (or 2% Triton X-100 where indicated), 150 mM NaCl, 10 mM iodoacetamide, 20 mM Hepes (pH 7.8), phosphatase inhibitors (1 mM sodium orthovanadate, 50 mM sodium fluoride), and protease inhibitors (16). The insoluble debris was removed by centrifugation at 14,000 rpm in an Eppendorff microcentrifuge for 15 min at 4°C. The supernatant was cleared three times by incubation for at least 1 hr with 25 μl of staphylococcal protein A-Sepharose [final concentration 50% (vol/vol), preincubated with normal hamster serum for 1 hr and washed three times in lysis buffer]. The protein A-absorbed cell lysates were divided into aliquots corresponding to $25–50 \times 10^7$ cells for immunoprecipitation with several mAbs. After 2-hr to overnight incubation at 4°C, immunoprecipitates were washed three times with 0.5% Brij-35 or 0.5% Triton X-100, 150 mM NaCl, in 20 mM Hepes (pH 7.4); twice with 0.1% Brij-35 or 0.1% Triton X-100, 150 mM NaCl, in 20 mM Hepes (pH 7.4); and once with 150 mM NaCl in 20 mM Hepes (pH 7.4). The immunoprecipitates were resuspended in SDS sample buffer and frozen at −70°C. Samples were analyzed by SDS/12.5% PAGE.

Immunoblotting of CD3-ζ and CD3-ε. Western blotting experiments were done on TCR-β or CD3-ε as described previously (15). The blotting antibodies were an anti-peptide antibody, N39, specific for CD3-ζ, or an affinity-purified anti-CD3-ε anti-peptide antibody (Dako), both at 1:2000 dilution. This was followed by washing with TBS-T [10 mM Tris·HCl-buffered (pH 7.5) 150 mM NaCl containing 0.1% Tween-20] and development with a goat anti-rabbit IgG conjugated with alkaline phosphatase (Promega), at 1:7500 dilution.

RESULTS

TCR × p53 or RAG-1 × p53 Double Mutant Mice Develop Thymic Lymphomas at High Frequency. Mice homozygous for a mutation in the TCR-α, TCR-β, or RAG-1 gene were crossed with p53 homozygous mutant mice. The double mutant mice were healthy for several months, after which most of them developed one or more tumors. More than two thirds of the tumors were thymic lymphomas. Attempts to establish thymic lymphomas in cell culture were successful in the majority of cases.

Expression of various surface markers on the cell lines was evaluated by flow cytometric analysis. For comparison of the expression levels, we included in our analysis the hybridoma 3DO-58.4 and the thymic lymphoma EL-4. Fig. 1 shows staining profiles of θ6b, derived from a p53 mutant mouse, and cell lines from five different TCR-α × p53 double mutant mice, θ4c.1, θ37b, θ9a, θ22b, and θ26c. All the lymphomic cell lines derived from TCR-α × p53 double mutant mice (hereafter called "TCR-α mutant cell lines") expressed CD3-ε and TCR-β at high levels. The expression levels were comparable to those of 3DO-58.4 and EL-4, and to θ6b, a thymoma cell line derived from a p53 mutant mouse (Fig. 1). The presence of the events on the diagonal in two-color analysis indicates that CD3-ε and TCR-β are expressed in approximately stoichiometric amounts. Using a panel of TCR-Vβ-specific monoclonal antibodies (against TCR-Vβ-2, -3, -5, -6, -7, -8, -9, -10, -11, -12, and -13), we were able to determine the TCR-Vβ usage in some lines (see Fig. 1). In these cases, the appearance of the dots on the diagonal in a staining for CD3-ε and the relevant TCR-Vβ chain (data not shown) suggests, but does not prove, that the cell line is monoclonal. Since all TCR-α mutant lines did not stain for GL3, a mAb against TCR-δ, it is unlikely that the TCR-β chain is part of a TCR-βδ heterodimer (17). Most of the thymic lymphomas and the cell lines were $CD4^+CD8^+$ (data not shown), including those derived from RAG-1 mutant mice. Some p53 mutant lymphomas or TCR-α × p53 double-mutant lymphomas contained a high proportion of $CD4^-CD8^+$ cells, and some RAG-1 × p53 double mutant lymphomas contained a high proportion of $CD4^+CD8^-$ cells (data not shown).

Association of CD3-ζ and CD3-ε with TCR-β in TCR-α Mutant Cell Lines. Stable pairwise assembly has previously been observed between single TCR-α or TCR-β chains and CD3 chains (18). In contrast, the CD3-ζζ dimer assembles more efficiently with the TCR chains when all of the other CD3 components are present (16, 18–20). To determine whether

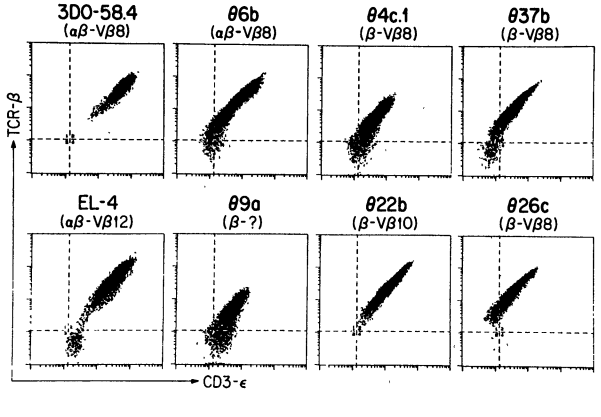

FIG. 1. Flow cytometric analysis of the lymphomic cell lines. Horizontally, CD3-ε (145-2C11-FITC), vertically, TCR-β (H57-597-PE). The left profiles in both rows are control cell lines: the hybridoma 3DO-58.4 and the thymic lymphoma EL-4. θ6b is a cell line derived from a p53 mutant mouse; this cell line presumably expresses a conventional TCR-αβ heterodimer, since two bands in the 45-kDa range are immunoprecipitated with H57-597 and cytosmears are brightly stained with H28-760 (an anti-TCR-α mAb) (data not shown). The other five cell lines are derived from TCR-α × p53 double mutant mice and therefore express only TCR-β (indicated below the name of the cell line in parentheses). The TCR-Vβ chain usage could be determined in all but one case with the use of a panel of mAbs.

the CD3-ζ chain was associated with the TCR-β chain in the TCR-α mutant cell lines, cells were solubilized in a buffer containing 1% Brij-35, a detergent which is sufficiently weak to maintain the integrity of the TCR-CD3 complex. Cell lysates were immunoprecipitated with H57-597, a mAb recognizing all TCR-β chains. The immunoprecipitates were separated with SDS/PAGE under nonreducing conditions, and Western blotting was carried out with N39, an anti-CD3-ζ-peptide serum (Fig. 2*A*). A band of molecular mass around 32 kDa representing CD3-$\zeta\zeta$ dimers can be seen in the four p53 mutant lines (lanes 6, 7, 8, and 12) and the six TCR-α mutant lines (lanes 1, 2, 3, 4, 5, and 11). The CD3-ζ chain was not present in the H57-597 immunoprecipitates in the TCR-β mutant line (lane 9) and the RAG-1 mutant line (lane 13): indeed, these cells are genetically unable to produce TCR-β chains. To determine the amount of CD3-ε coprecipitable with the TCR-β chain, the bottom part of the same blot was probed with an anti-CD3-ε peptide antibody. CD3-ε was coimmunoprecipitated with the H57-597 antibody in the p53 mutant and TCR-α mutant cell lines, but, as expected, not in the TCR-β mutant (lane 9) and in the RAG-1 mutant cell line (lane 13). These results strongly suggest that all six TCR-α mutant lines analyzed contained complexes consisting of TCR-β assembled with either CD3-ζ or CD3-ε, or both.

Association of CD3-ζ with CD3-ε Chains. To determine whether the CD3 components could be associated with the CD3-ζ chain in the absence of TCR-α and/or TCR-β chains, cell lysates from the same cell lines described above were immunoprecipitated with 145-2C11, a mAb against CD3-ε that recognizes a conformational epitope formed by CD3-$\varepsilon\delta$ or CD3-$\varepsilon\gamma$ dimers. The 145-2C11 immunoprecipitates were subject to nonreducing SDS/PAGE and Western blotting with N39 (Fig. 2*C*). A band of about 32 kDa, representing CD3-$\zeta\zeta$ dimers, could be seen in all the samples (four p53 mutant lines, six TCR-α mutant lines, one TCR-β mutant line, and one RAG-1 mutant line), except for the preclearing sample of θ30b (lane 10). The bottom part of the filter was probed with the anti-CD3-ε peptide serum (Fig. 2*D*). Similar amounts of CD3-ε were immunoprecipitated from all the cell lysates. Thus, the CD3-ζ chain is associated with CD3-ε in the thymoma cell lines, irrespective of the presence of either the TCR-α or the TCR-β chain.

Surface Expression of CD3 Components in the RAG-1 Mutant Line. The previous experiments did not allow us to discriminate between intracellular and surface expression of the complexes. In particular, we wished to know whether in the RAG-1 mutant line θ8c, CD3-ε was also expressed on the cell surface. Flow cytometric analysis gave a signal that was not convincingly above background (data not shown). Therefore, surface labeling was carried out by using the ^{125}I-lactoperoxidase method, the cell lysates were immunoprecipitated with 145-2C11, and the precipitates were analyzed by two-dimensional nonreducing vs. reducing SDS/PAGE. Autoradiography allowed visualization of the proteins coprecipitated with CD3-ε. After prolonged exposure, other than the off-diagonal spot representing CD3-ε, two coprecipitating pro-

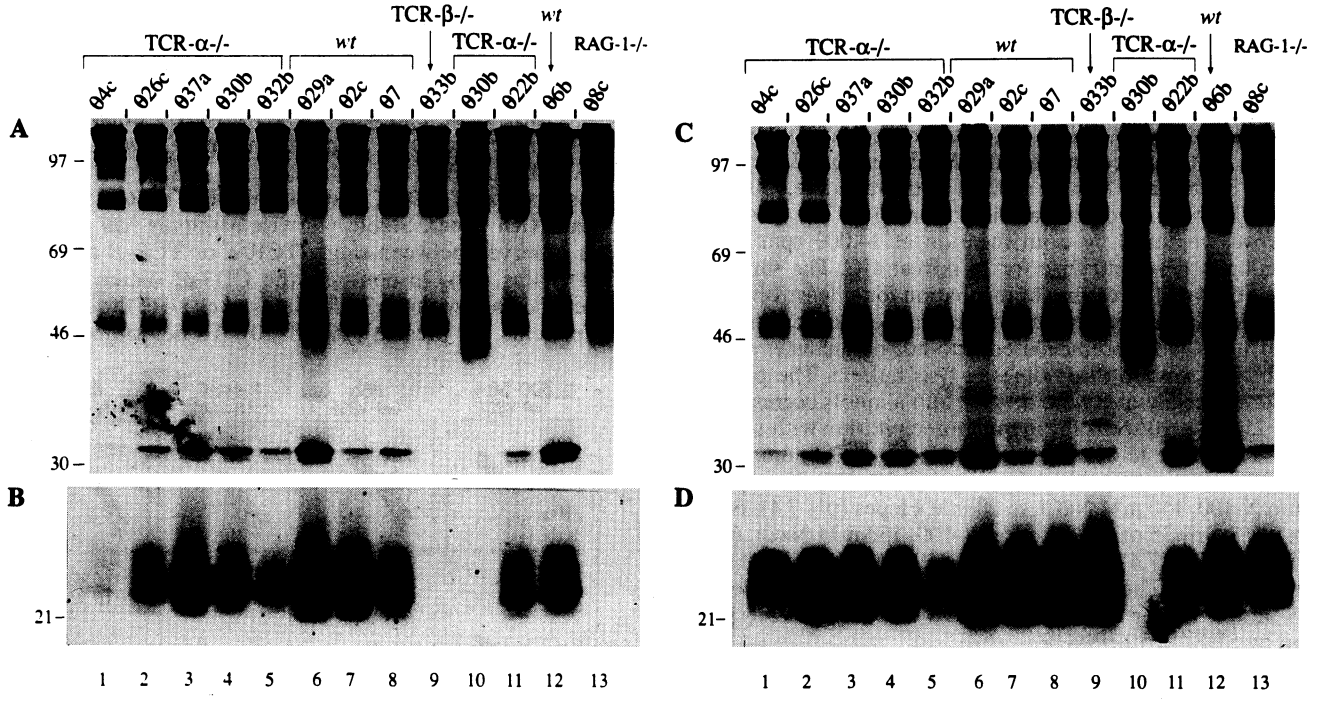

FIG. 2. Lysates (1% Brij-35) of various cells were immunoprecipitated and then subjected to Western blotting. *A* and *B* are upper and lower parts of the same gel, and *C* and *D* are upper and lower parts of another gel. Samples were derived from the same total cell lysates. SDS/PAGE was under nonreducing conditions. Numbers on left are positions of markers in kDa; *wt*, wild-type (for TCR genes and RAG-1); TCR-α $-/-$, TCR-α mutant; TCR-β $-/-$, TCR-β mutant; RAG-1 $-/-$, RAG-1 mutant. (*A* and *B*) Association of CD3-ζ and CD3-ε with TCR-β in TCR-α mutant cell lines. (*A*) Immunoprecipitation with anti-TCR-β mAb H57-597 followed by Western blotting with anti-CD3-ζ antiserum N39. CD3-ζ is coprecipitated in all lines except the TCR-β mutant line θ33b (lane 9) and the RAG-1 mutant line θ8c (lane 13). Lane 10 is a preclearing sample of TCR-α mutant line θ30b. (*B*) Immunoprecipitation with anti-TCR-β mAb H57-597 followed by Western blotting with an anti-CD3-ε peptide antibody. Similar amounts of CD3-ε are coprecipitated with TCR-β in wild-type and in TCR-α mutant cell lines. As expected, CD3-ε is not coprecipitated from the TCR-β mutant (lane 9) and RAG-1 mutant (lane 13) cell lines. (*C* and *D*) Association of the CD3-ζ chain with CD3-ε. (*C*) Immunoprecipitation with anti-CD3-ε mAb 145-2C11 followed by Western blotting with anti-CD3-ζ antiserum N39. The band at the bottom, around 32 kDa, corresponds to the CD3-ζ homodimer. The two bands just above the CD3-ζ dimer (lanes 6–9 and 12) may represent posttranslational modifications of CD3-ζ. The bands above the 46-kDa marker represent nonspecifically precipitated proteins. The ζ chain is coprecipitated with CD3-ε in all cell lines (lane 10 represents a preclearing of line θ30b). (*D*) Immunoprecipitation with 145-2C11 followed by Western blotting with the anti-CD3-ε peptide antiserum. Similar amounts of CD3-ε (bands above the 21-kDa marker) are immunoprecipitated from the total cell lysates, except for line θ32b, which may be underloaded. The preclearing of θ30b does not show any bands (lane 10).

teins were detected, which are most likely CD3-δ and CD3-γ on the basis of their characteristic localization on the diagonal, in the proximity of CD3-ε (Fig. 3).

Inasmuch as CD3-ζ is concerned, surface iodination cannot visualize this protein due to the absence of a tyrosine residue in the short extracellular domain of CD3-ζ. Surface labeling with an activated biotin ester, followed by two-dimensional electrophoresis of N39 immunoprecipitates, revealed CD3-ζ as a 16 kD spot off the diagonal, suggesting that the CD3-ζ chain indeed is present on the surface of the RAG-1, TCR-α, and TCR-β lines (data not shown).

DISCUSSION

Use of p53 Mutant Mice to Derive Thymocyte Cell Lines. We are interested in the molecular analysis of partial CD3–TCR complexes on the surface of immature thymocytes, at stages prior to expression of the conventional TCR-αβ heterodimer. As the number of thymocytes is very small in RAG-1 mutant mice, and the expression level of CD3-ε and TCR-β is very low in TCR-α mutant mice, we established cell lines from the mutant mice and used them for the analysis, rather than thymocytes isolated from the mutant mice. We chose not to produce T-cell hybridomas, as the fusion partner itself is a thymic lymphoma and the fusion may create artefactual expression patterns or cellular properties. Instead, we crossed mice with mutations in RAG-1, TCR-α, or TCR-β, to p53 mutant mice, which were previously known to develop DP thymic lymphomas at high frequency. A description of the tumor spectrum in these double mutant mice will be provided elsewhere (P.M., T.J., B. Williams, R. Bronson, and S.T., unpublished work).

The thymic lymphomas were exclusively of one type: DP cells, expressing, when genetically possible, TCR-β at high levels and in an approximately stoichiometric amount compared with CD3-ε. The expression level of TCR-β on the surface of the TCR-α mutant lines was much higher than on TCR-α mutant thymocytes. Possible explanations are the large difference in size between the lymphomic cells and normal thymocytes (data not shown), the vigorous growth and metabolic activity of the lymphomic cells compared with thymocytes, or the transformed nature of the lymphomic cells. The stoichiometric expression of a particular TCR-Vβ region and the CD3-ε chain suggests that the thymic lymphomas derived from p53 mutant mice or TCR-α × p53 double mutant mice are monoclonal. Proof for the clonality awaits cloning and sequencing of all rearranged TCR genes of these cell lines.

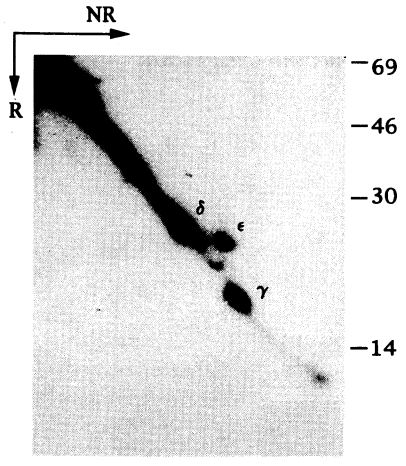

FIG. 3. Surface expression of the CD3 chains in the RAG-1 mutant line θ8c. Cells were iodinated with the ^{125}I–lactoperoxidase method and lysed in the presence of 1% Brij-35 detergent. Immunoprecipitation was carried out with 145-2C11, followed by two-dimensional SDS/PAGE. Vertically, reducing, horizontally, nonreducing conditions. The autoradiograph was exposed for 7 days at −80°C. The two spots on the diagonal close to CD3-ε most likely correspond to CD3-δ and CD3-γ.

p53 mutant mice may be useful for obtaining with relative ease cell lines from thymocytes of other genetically manipulated mice (21). Our results indicate that neither TCR expression nor V(D)J rearrangement is a prerequisite for the development of thymic lymphomas in a p53 mutant background. Caution is required to interpret findings obtained with these cell lines (as with all other transformed lines). In particular, we draw attention to the expression of CD4 and CD8 in thymic lymphomas from RAG-1 × p53 double mutant mice, which is unexpected in view of the absence of DP cells in the RAG-1 mutant thymus. A possible explanation is tumor-related deregulation of CD4 and CD8 gene expression.

Expression of Immature CD3–TCR Complexes. Our flow cytometric and biochemical analysis has revealed the presence of partial CD3–TCR surface complexes on immature thymocyte lines: a complex containing CD3-ε, -γ, -δ, and -ζ in the RAG-1 mutant line and a complex containing CD3-ε, -γ, -δ, and -ζ and TCR-β in the TCR-α mutant lines.

There are indications that these observations reflect the *in vivo* situation. First, surface expression of signal transducing-competent, CD3-ε-containing complexes in RAG-1 mutant thymocytes has been inferred from the observation that addition of the anti-CD3-ε mAb 145-2C11 to RAG-1 mutant fetal thymic lobes resulted in the appearance of large numbers of DP thymocytes (22). Second, TCR-β is detectable by flow cytometry at very low levels on a fraction of TCR-α mutant thymocytes (7, 9, 23); this TCR-β surface-positive fraction of TCR-α mutant thymocytes is distinguishable from the bulk of thymocytes by its larger size (9, 23). Since all our TCR-α mutant lines express TCR-β on the surface, it is conceivable that the absence of p53 preferentially leads to transformation of large pre-T cells. Third, participation of CD3-ζ in early thymocyte differentiation can be deduced from the severe reduction in the number of DP thymocytes in CD3-ζ mutant mice (24–27).

Comparison with Other Pro-T or Pre-T Cell Lines. The expression of immature CD3–TCR complexes on thymocyte cell lines is not unprecedented. SL12.4 and SL12.1 are thymomic cell lines that express CD3 components without TCR chains on the cell surface (28). KKF is a Gross leukemia virus-transformed DP thymocyte line, expressing on the surface CD3-ε, -γ, and -δ, TCR-β, and, in weak association, also CD3-ζ (29). Sci/ET27F is a DN line that spontaneously arose in culture from the thymus of a severe combined immunodeficiency (*scid*) mouse (30). This cell line was reported to express low levels of CD3-ζ on the surface, but no CD3-ε. After stable transfection of Sci/ET27F with a rearranged TCR-β gene, TCR-β chains could be detected on the surface of transfectant SCB.29 that were tightly associated with CD3-ε and CD3-δ and weakly associated with CD3-γ and CD3-ζ. Originally, the TCR-β chains were reported to be homodimers (30), but, by using surface biotinylation or metabolic labeling with [^{3}H]leucine, the TCR-β chains were shown to be disulfide-linked to a 33-kDa glycoprotein (called gp33) (9).

The physiological significance of the expression of partial CD3–TCR complexes in these lines (28–30) has not yet been established. However, in light of the growing evidence for CD3-ε and CD3/TCR-β-containing complexes on the surface of pro-T cells and pre-T cells, respectively, some of these cell lines may now be regarded as representatives of such early thymocytes. KKF can be considered as the first example of a pre-T cell line. The particular value of our mutant cell lines is that their genetic origin is clearly defined.

Surface Expression of CD3-ζ Without TCR-α. The surface expression of CD3-ζ in cells that are unable to express TCR-α chains is in contradiction to a number of studies implicating a

requirement for the formation of a TCR-$\alpha\beta$ heterodimer prior to the integration of CD3-ζ into the TCR-CD3 complex, which is generally regarded as the last step in CD3–TCR assembly (3, 16, 20, 31). It should be taken into account, however, that those data were obtained by using mature T-cell lines, from which mutants were derived that were then rescued by transfection of CD3 or TCR components, or by transfecting CD3 and TCR genes into nonlymphoid cells. The paradox can be resolved by assuming the presence of stage-specific and as-yet-unknown molecules in the assembly of immature CD3–TCR complexes. The rules for assembly and transport to the surface may be different in immature T cells compared with the well-studied mature T-cell lines. One of these uncharacterized proteins could be gp33 (9); other proteins may be chaperones or even a surrogate TCR-β chain.

It has not escaped our notice that surface expression of CD3-ε- or TCR-β-containing complexes on early thymocytes may not be physiologically relevant. Indeed, it may be the result of some kind of leakage. To date there is no proof that these chains need to be expressed on the cell surface for signal transduction to occur. Intracellular signaling events could be sufficient.

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