T CELL RECEPTOR γ/δ CHAIN DIVERSITY

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A second type of TCR composed of a γ gene product and a putative δ gene product has been identified on subpopulations of human and murine thymocytes (1, 2), human lymphocytes (3–5), and murine Thy-1$^+$ skin cells (6, 7). Although some TCR-γ/δ-expressing cells can exert NK cell activity in vitro (3–5) that can be blocked by anti-T3 (3–5) and antiidiotypic reagents (5), the ligand and the physiological role for the TCR-γ/δ remains unclear.

It is important to gain insight into the diversity of the TCR-γ/δ to define its recognitive potential. The γ chain gene family is known to encode four C regions (1 pseudogene) and seven V regions which can give rise to at least five γ chain mRNA transcripts (8–13). It is unknown if all these transcripts give rise to cell surface–expressed proteins. Recently, a possible candidate for the δ gene family has been identified (14), but little data exist concerning the diversity of the expressed protein. To investigate the diversity of TCR-γ/δ at the protein level, we compared TCR-γ/δ from a panel of six murine hybridomas, two derived from C3H/HeN Thy-1$^+$ dendritic epidermal cells (15) and four from C57BL/6 thymocytes (16).

Materials and Methods

Cell Lines. T cell hybridomas expressing TCR-γ/δ were made by fusion of C3H/HeN-derived Thy-1$^+$ dendritic epidermal cell lines (15) or cultured C57BL/6-derived Lyt-2$^-$,L3T4$^-$ thymocytes (16) with the BW5147 fusion partner as described previously (15, 16). These hybridomas express the CD3-associated TCR-γ/δ (15, 16).

Immunoprecipitation, SDS-PAGE, and NEPHGE-analysis. Immunoprecipitations were carried out using the anti-T3 mAb 2C11 (17) and ^{125}I-labeled digitonin lysates of TCR-γ/δ expressing hybridomas as described previously (6). Immunoprecipitates were either analyzed on 12% SDS-PAGE gels or on two-dimensional NEPHGE/SDS-PAGE gels. For NEPHGE (18), the immunoprecipitates were resuspended in 50 μl sample buffer (9 M urea, 2% NP-40, 2% ampholines, pH 3.5–10 [LKB, Gaithersburg, MD], 5% 2-Mercaptoethanol) and applied to 12-cm NEPHGE cylindrical gels (4% acrylamide, 2% NP-40, 5% ampholines pH 3.5–10 [LKB] 9 M urea). The gels were run for 6 h at 500 V. After a 2-h equilibration of these cylindrical gels with SDS-PAGE sample buffer (125 mM Tris-HCl, 10% glycerol, 5% 2-ME, 2.3% SDS), they were applied to 12% SDS-PAGE gels for electrophoresis in the second dimension. Gels were dried and autoradiography was carried out at −70°C.

N-Glycanase and Neuraminidase Treatment. Removal of N-linked sugars was performed using N-glycanase (Genzyme, Boston, MA) as described previously (6). For removal of
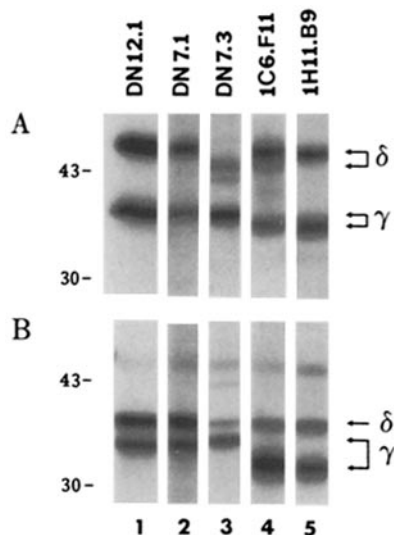
FIGURE 1.   Comparison of the TCR-γ/δ expressed by a panel of five hybridomas. Cells were surface iodinated and, after lysis, the TCR-γ/δ-T3 complexes were isolated by precipitation with the anti-T3ε mAb 2C11. Duplicate precipitations were carried out and one of these was treated with N-glycanase to remove N-linked sugars. All precipitates were analyzed on SDS-PAGE gels under reducing conditions. (A) untreated, (B) N-glycanase treated. γ and δ chains of: (1) DN12.1, (2) DN7.1, (3) DN7.3, (4) 1C6.F11, and (5) 1H11.B9 are shown.

sialic acid residues, immunoprecipitates were incubated for 16 h at 37°C with 0.1 U neuraminidase (No. N5631; Sigma Chemical Co., St. Louis, MO) in 50 mM sodium acetate buffer, 0.9% NaCl, 0.1% $CaCl_2$, pH 5.5.

Results and Discussion

Six TCR-γ/δ-expressing hybridomas (1C6.F11 and 1H11.B9 of C3H/HeN origin and DN12.1, DN7.1, DN7.3, and DN2.3 of C57BL/6 origin) were used in this study. Earlier studies (2, 6, 12, 13) have indicated that all these hybridomas express glycosylated γ chains that are not derived from Vγ-Cγ4 transcripts since the antiserum used to detect these γ chains does not react with Cγ4 chains (nomenclature according to Garman et al., [10]). Furthermore, since the Cγ3 locus is a pseudogene (8) and, at least for the C57BL/6 mice, Vγ1.2-Cγ2 transcripts would result in nonglycosylated γ chains (19), these hybridomas most likely express γ chains derived from Vγ-Cγ1 transcripts.

These hybridomas were ^{125}I-labeled and subsequently lysed in digitonin buffer. From these lysates the TCR-γ/δ–T3 complexes were isolated by immunoprecipitation with the anti-T3ε mAb 2C11. The anti-T3 precipitates were digested with N-glycanase, which specifically removes N-linked sugars, and these digested samples and control, undigested samples were subsequently analyzed on SDS-PAGE. The results (Fig. 1) indicate that the γ chains of both C3H/HeN-derived hybridomas (1C6.F11 and 1H11.B9) have a slightly lower molecular weight (both before and after removal of N-linked sugars) than the γ chains of the C57BL/6 derived hybridomas. A small but reproducible difference in the mobility of the DN7.3 γ chain versus the DN7.1 and DN12.1 γ chains was also observed (Fig. 1; data not shown). The C57BL/6 derived hybridomas DN7.3 and DN2.3 expressed a δ chain (glycosylated) with a lower molecular weight than that of the δ chains expressed by the other hybridomas (results shown for the DN7.3 hybridoma only). No clear differences in the molecular weights of deglycosylated δ chains could be observed.

Next, anti-T3 precipitates of the ^{125}I-labeled lysates were analyzed in more
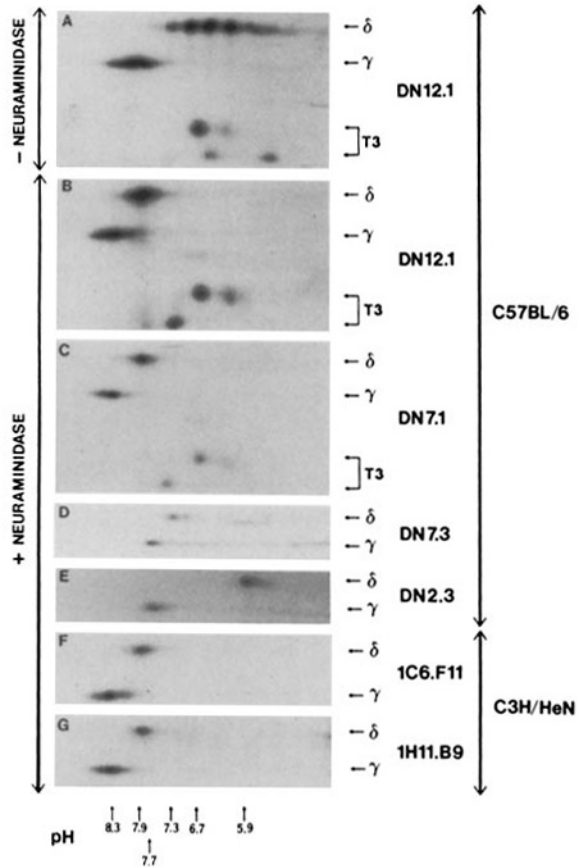
FIGURE 2. Two-dimensional gel analysis (2-D NEPHGE/SDS-PAGE) of TCR-γ/δ chains. Receptor complexes were isolated by immunoprecipitation with the anti-T3ϵ mAb and analyzed on two-dimensional NEPHGE/SDS-PAGE as described in Materials and Methods. NEPHGE was horizontal from right to left and SDS-PAGE was vertical from top to bottom. pH values indicated were calculated from measurements in 1-cm sections of a NEPHGE tube gel, which was run in parallel to the other samples. Gels were aligned using the loading point of the NEPHGE gels and the T3 components as invariant spots. In the case of panels D and E, overexposed gels (not shown) were used to verify the location of the T3 components. A non–neuraminidase-treated sample was analyzed in A. Samples in B–G were all neuraminidase treated. TCR chains from the following hybridomas were analyzed: (A and B) DN12.1, (C) DN7.1, (D) DN7.3, (E) DN2.3, (F) 1C6.F11, and (G) 1H11.B9.

detail by two-dimensional NEPHGE/SDS-PAGE (Fig. 2). To remove charge heterogeneity due to sialic acid residues, duplicate precipitates were incubated for 16 h at 37°C with or without neuraminidase. Comparison of a neuraminidase-treated sample (Fig. 2B) with a non–neuraminidase-treated sample (Fig. 2A) illustrates the effective reduction in charge heterogeneity by the removal of sialic acids. With all γ and δ chains analyzed in this panel, a shift to the basic side of the gels was observed after neuraminidase treatment, indicating the presence of sialic acid residues on all these γ and δ chains (results before neuraminidase treatment are shown for the DN12.1 TCR-γ/δ only). To allow comparison of the immunoprecipitates, the gels were aligned using the T3 components (as shown in Fig. 2, A–C) and the loading point of the NEPHGE gels (not shown) as invariant internal spots. The proper alignment of γ and δ chains was crosschecked in two additional experiments (not shown). The results show that the γ and δ chains of two C57BL/6-derived hybridomas (DN12.1 and DN7.1, Fig. 2, B and C) are indistinguishable by size and charge, and differ from those of both the DN2.3 and DN7.3 hybridomas (Fig. 2, D and E). The δ chains of the DN2.3 and DN7.3 hybridomas are significantly different from each other (pH 7.3 vs. pH 5.9), whereas the γ chains are similar or identical. Thus the C57BL/6 hybridomas express three distinguishable δ chains and two distinguishable γ chains. Since the

TABLE I

Characteristics of γ and δ Chains Examined

Strain		Molecular weight ($\times 10^{-3}$)		Charge (pH)*	Expressed	
		Glycosylated	Non-glycosy-lated		by	with
C57BL/6	γ_a	35	32	8.3	DN12.1	$\delta_a^\ddagger$
					DN 7.1	$\delta_a^\ddagger$
	γ_b	35	32.5	7.7	DN7.3	δ_b
					DN2.3	δ_c
	$\delta_a^\ddagger$	45	35	7.9	DN12.1	γ_a
					DN 7.1	γ_a
	δ_b	44	35	7.3	DN 7.3	γ_b
	δ_c	44	35	5.9	DN 2.3	γ_b
C3H/HeN	γ_c	34	31	8.3	1C6.F11	$\delta_a^\ddagger$
					1H11.89	$\delta_a^\ddagger$
	$\delta_a^\dagger$	45	35	7.9	1C6.F11	γ_c
					1H11.B9	γ_c

* Since the γ and δ chains were analyzed by nonequilibrium pH gradient electrophoresis, the chains do not run to their true isoelectric point. Therefore, the pH values given indicate only charge differences between the various γ and δ chains and do not represent pI values.

‡ Although the δ_a chains expressed by the C57BL/6 and C3H/HeN hybridomas are indistinguishable, it is not clear whether they are truly identical.

γ chains from the C57BL/6 hybridomas are most likely derived from Vγ-Cγ1 transcripts (see above), the difference in isoelectric points between these γ chains (DN2.3,DN7.3: pH 7.7; DN7.1,DN12.1; pH 8.3) may reflect the use of different V regions. The γ and δ chains expressed by the independently derived C3H/HeN hybridomas are indistinguishable by size and charge (Fig. 2, F and G). Furthermore, these chains are identical in charge to the γ and δ chains expressed by the DN7.1 and DN12.1 hybridomas (Fig. 2, B and C).

To verify that the observed electrophoretic mobilities were due solely to primary sequences, we examined the γ and δ chains for the presence of potential charged prosthetic groups beside sialic acid. Essentially identical results to that obtained with neuraminidase treatment alone were obtained when N-glycanase[R] or N-glycanase[R] plus neuraminidase were used to remove charge due to the presence of carbohydrate side chains (not shown). This indicated that such charge heterogeneity was due entirely to the presence of sialic acid residues. To rule out phosphorylation as a source of charge heterogeneity, we labeled with radioactive phosphate. Neither the γ nor δ chains incorporated phosphate; however, the T3ε chain was labeled as expected (Pardoll, D. N., and D. Meuller, unpublished observations). Also the γ or δ chains did not incorporate [^{35}S]sulfate (not shown) ruling out sulfation as a source of heterogeneity.

The results presented in this study clearly indicate that, like γ chains, multiple forms of the δ chain exist. Three distinct δ chains could be identified that were found to form four different γ/δ chain combinations with three distinct γ chains (Table I). Although three different TCR-γ/δ could be easily distinguished on the four C57BL/6 thymus derived hybridomas (DN7.1, DN12.1, DN7.3, and DN2.3), it is striking that the two independently derived hybridomas of C3H/HeN origin (1C6.F11 and 1H11.B9), which are both from the periphery (skin), express an identical receptor. Also, the δ chain expressed on the C3H/HeN-derived hybridomas is indistinguishable in charge from that expressed

on the C57BL/6-derived hybridomas DN7.1 and DN12.1 (Fig. 2, Table I). Therefore, although our results demonstrate that δ chain diversity exists (three different δ chains detected), this diversity is apparently quite limited (four of six hybridomas express an apparently identical δ chain).

In conclusion, our results indicate that in addition to the γ chains, the δ chains display diversity, even among hybridomas derived from a homozygous inbred strain. This also indicates that the δ chain could be encoded for by the recently described TCR gene family, which is located within the α locus (14), a notion that is also supported by the recent observation that putative human δ cDNA clones encode a protein that can be precipitated by an anti-δ mAb (20, 21). Moreover, the δ chain diversity may significantly increase the polymorphic potential of γ/δ receptors; however, further investigations will be needed to clarify if this δ chain diversity arises from the differential use of V, C, D, and/or J-regions. Finally, screening of a larger panel of both thymus- and peripheral-derived TCR-γ/δ expressing cells from one mouse strain will be needed to clarify if thymus selection of, or thymus selection against certain TCR-γ/δ influences the repertoire of TCR-γ/δ expression in the periphery.

Summary

The TCR-γ and -δ chains of six murine hybridomas were compared by one-dimensional SDS-PAGE and two-dimensional NEPHGE/SDS-PAGE analysis. This allowed the identification of three distinct γ chains (γ_a, γ_b, and γ_c) and three distinct δ chains (δ_a, δ_b, and δ_c). Four γ/δ chain combinations ($\gamma_a\delta_a$, $\gamma_b\delta_b$, $\gamma_b\delta_c$, and $\gamma_c\delta_a$) were observed. These results indicate that multiple forms of the δ chain are expressed and suggest that the δ chains are encoded for by an Ig-like rearranging gene. This δ chain polymorphism significantly enhances the potential diversity of TCR-γ/δ, which may be of importance for a better understanding of the putative ligand(s) recognized by this receptor.

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