Brief Definitive Report

PRODUCTION OF A T CELL HYBRIDOMA THAT EXPRESSES THE T CELL RECEPTOR γ/δ HETERODIMER

By WAYNE M. YOKOYAMA,* FRITS KONING,‡ GEORG STINGL,‖ JEFFREY A. BLUESTONE,§ JOHN E. COLIGAN,‡ AND ETHAN M. SHEVACH*

*From the Laboratories of *Immunology and ‡Immunogenetics, National Institute of Allergy and Infectious Diseases, the §Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892; and the ‖Department of Dermatology I, University of Vienna, Vienna, Austria*

MHC-restricted antigen recognition is mediated by a T cell receptor (TCR) composed of a disulfide-linked heterodimer of the α and β chains and the multicomponent T3 complex (1). Recently, a second T3-associated putative TCR heterodimer has been described on a subset of human PBL (2–4), thymocytes (5), a leukemia line (6), and on a subset of murine thymocytes (7–9). This second TCR is composed of the protein products of rearranged TCR γ-chain genes, which are associated with a poorly characterized chain termed δ. We have recently demonstrated by immunofluorescent staining of epidermal sheets that a population of Thy-1$^+$, bone marrow–derived, epidermal cells with a dendritic morphology (Thy-1$^+$ DEC) express T3 and are also reactive with an antiserum to the TCR γ chain (10).[1] Long-term IL-2-dependent cell lines derived from Thy-1$^+$ DEC express a T3-associated 34 kD γ chain that is frequently linked to a 46 kD partner.[2]

In the present report, we describe the initial characterization of a T cell hybridoma derived from the fusion of a lymphokine-dependent long-term TCR γ/δ^+ Thy-1$^+$ DEC cell line with the BW5147 tumor line. The resultant hybridoma is rapidly growing, lymphokine independent, expresses T3 in association with the TCR γ and δ chains, and secretes IL-2 in response to stimulation with anti-T3 and Con A.

Materials and Methods

Cell Lines. The conditions for the establishment and maintenance of the Thy-1$^+$ DEC cell line, T245, have been described previously (10). The HAT-sensitive BW5147 T cell line was obtained from the American Type Culture collection (ATCC), Rockville, MD.

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[1] Stingl, G., F. Koning, H. Yamada, W. M. Yokoyama, E. Tschachler, J. A. Bluestone, G. Steiner, L. E. Samelson, A. M. Lew, J. E. Coligan, and E. M. Shevach. Thy-1$^+$ dendritic epidermal cells express T3 and the T cell receptor gamma chain. *Proc. Natl. Acad. Sci. USA.* In press.

[2] Koning, F., G. Stingl, W. M. Yokoyama, H. Yamada, W. L. Maloy, E. Tschachler, E. M. Shevach, and J. E. Coligan. Identification of a T3-associated γ/δ T cell receptor on Thy-1$^+$ dendritic epidermal cell lines. *Science (Wash. DC).* In press.

The 2B4 T cell hybridoma, a BW5147 fusion product which expresses TCR α/β (11), was obtained from Dr. Jonathan Ashwell (NIH).

Production of T Cell Hybridomas. A 1:1 mixture of T245 and BW5147 cells was stirred in 50% (wt/vol) PEG (E. Merck, Darmstadt, Federal Republic of Germany) for 2 min, washed, and plated ($2–4 \times 10^5$ cells/well) in 96-well Costar plates (Costar, Cambridge, MA) containing 5×10^4 X-irradiated (3,000 rad) peritoneal "wash out" cells in 0.2 ml of RPMI 1640 containing 20% FCS, HAT, L-glutamine, penicillin/streptomycin, 2-ME, and Hepes buffer. After 11 d, hybridomas were picked, expanded, and tested for T3 expression by flow cytometry. Positive lines were cloned by limiting dilution (0.3 cells/well) in 96-well microtiter plates.

Antisera and mAbs. The properties of monoclonal hamster anti–mouse T3 ϵ antibody (145-2C11, 12), rabbit antisera to the mouse T3 δ chain (R9 kindly provided by Dr. Lawrence Samelson, NIH) (13) and to the mouse TCR γ chain (7) have been previously published.

Cell Surface Labeling, Immunoprecipitation, and SDS-PAGE. Cell surface labeling and immunoprecipitation were carried out as described previously (7, 14). Samples were analyzed on 12% polyacrylamide slab gels under either nonreducing or reducing conditions.

Assay of T Hybridomas for Activity. 10^5 T hybridoma cells were cocultured with 10^4 irradiated (10,000 rad) LS B lymphoma cells (15) or 10^5 irradiated (3,000 rad) anti-Thy-1.2 + C′–treated C3H spleen cells in 0.2 ml of medium in the presence of 145-2C11 culture supernatant (1:10 or 1:100, final dilution) or Con A (10 μg/ml). After 24–36 h of culture, supernatants were collected and assayed for IL-2 content in a secondary culture with CTLL cells as described previously (16). The degree of stimulation was assayed by the incorporation of [^{3}H]thymidine into DNA. Results are expressed as mean cpm incorporated by triplicate cultures.

Results and Discussion

We have previously demonstrated that the Thy-1$^+$ DEC cell line, T245, expresses no TCR α chain mRNA, the truncated (1.0 kb) form of the TCR β chain mRNA, but abundant levels of normal-size TCR γ chain mRNA (10). Immunoprecipitation studies of surface-labeled cells with anti–T3 δ chain and anti–TCR γ chain antisera revealed a 34 kD γ chain that was disulfide linked to a 46 kD δ chain partner.[2] However, further structural and functional studies of the TCR γ/δ complex on the surface of T245 cells were hampered because of the slow in vitro growth of this cell line, which is dependent on high concentrations of Con A–stimulated rat spleen cell supernatants. We therefore immortalized this cell line by fusing it to the T3$^-$ BW5147 tumor line and selecting for positive growth in the presence of HAT and in the absence of lymphokines. Growing wells were then screened for surface T3 expression by reactivity with anti-T3 mAb (145-2C11) and flow cytometry. Hybridomas of interest were subcloned by limiting dilution and subjected to further analysis. Flow cytometric analysis also revealed that the hybridomas displayed uniform reactivity with both an anti-Thy-1.1 mAb and an anti-Thy-1.2 mAb, whereas these Thy-1 alleles are independently expressed on the AKR-derived BW5147 and C3H-derived T245 parent cell lines, respectively. All hybridoma lines were also L3T4$^-$, Ly-2$^-$, and Ia$^-$ (data not shown).

To analyze the biochemical properties of the T3-associated heterodimer on the hybridomas, the parental cell lines, several T3$^+$ hybridomas and one T3$^-$ hybridoma (2G8) were cell surface–radioiodinated and specific immunoprecipitations were performed. No specific bands were seen when lysates of BW5147 or 2G8 were subjected to immunoprecipitation with the anti–T3 δ chain anti-

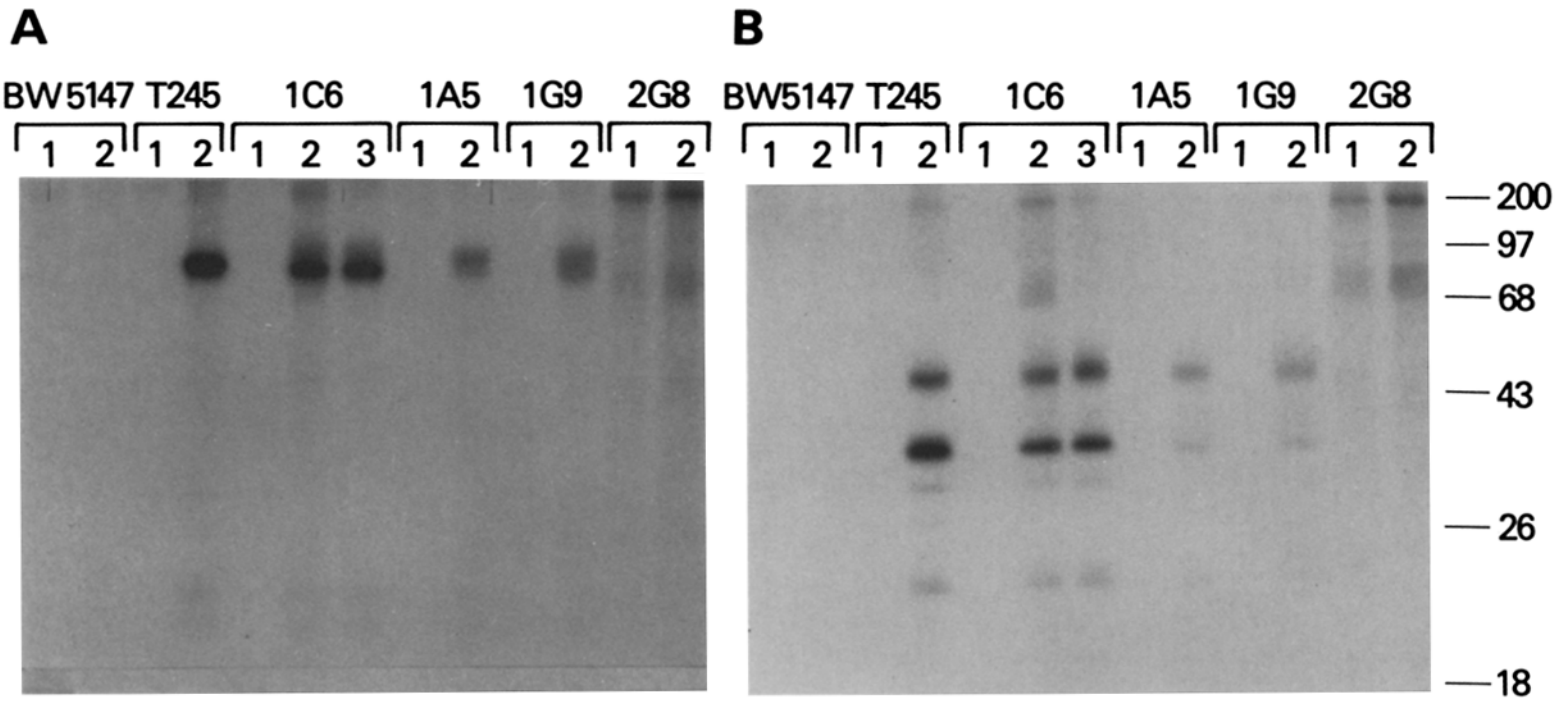

FIGURE 1. Analysis of T3 associated TCR molecules in ^{125}I-labeled digitonin lysates of the parental cell lines and several T cell hybridoma lines. Cells were surface iodinated, lysed in 1% digitonin and specific immunoprecipitations were carried out. SDS-PAGE was performed under nonreducing (*A*) or reducing conditions (*B*). Lanes *1*, control nonimmune rabbit serum; lanes *2*, anti–T3 δ antiserum (R9); lanes 3, anti–T3 ϵ mAb.

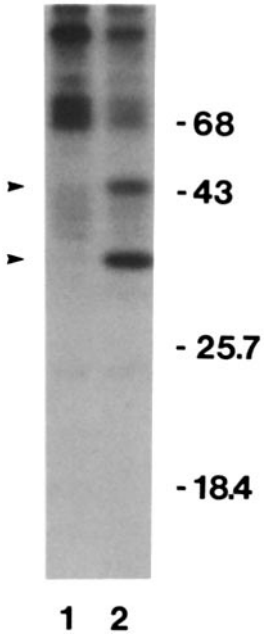

FIGURE 2. Analysis of TCR γ chain expression by the 1C6 cell line. 1C6 cells were surface iodinated, lysed in 1% NP-40, and specific immunoprecipitations were carried out. Lane *1*, normal rabbit serum; lane *2*, anti–TCR γ antiserum.

serum (Fig. 1,*A* and *B*). As previously shown,[2] the T245 cell line expressed a T3-associated 80–90 kD molecule under nonreducing conditions (Fig. 1 *A*), which was composed of 34 and 46 kD chains under reducing conditions (Fig. 1 *B*). All three $T3^+$ (1C6, 1A5, and 1G9) hybridomas expressed a 34–46 kD heterodimer that had the same mobility as that detected on T245 (Fig. 1 *B*). A similar complex was seen when immunoprecipitations were performed with the anti–T3 ϵ chain mAb 145-2C11 on lysates from the 1C6 hybridoma line (Fig. 1,*A* and *B*; 1C6, lane *3*). The 34–46 kD bands were also immunoprecipitated by the anti–TCR γ serum as shown in Fig. 2 for the 1C6 hybridoma proving that these hybridomas expressed the TCR γ/δ chains. Furthermore, the 34 kD band is the TCR γ chain, because in T245 lysates this chain has previously been demonstrated to react specifically with the anti-γ antiserum.[2] It should be noted that an additional band (~90 kD) is seen under nonreducing conditions in the immunoprecipitates of all three hybridoma lines that is not present in the precipitates of the T245 cell line (Fig. 1 *A*). It is unlikely that this higher-molecular-mass band is composed of the TCR α/β chains because no chains with the mobilities of the TCR α/β chains are seen in the reducing gel (Fig. 1 *B*). More detailed biochemical and molecular studies will be required to elucidate the origin of this molecular species.

To determine whether signal transduction may occur through the TCR γ/δ–T3 complex, the parental lines as well as two subclones of the $T3^+$ 1C6 cell line (1C6.F11 and 1C6.H10), and one subclone of the $T3^-$ T cell hybridoma (2G8.E8) were tested for their ability to produce IL-2 in response to stimulation with the anti-T3 mAb or the mitogen Con A. No IL-2 production was seen following stimulation of BW5147, T245, or the $T3^-$ hybridoma 2G8 with either Con A or anti-T3 (Table I). In contrast, both of $T3^+$, TCR γ/δ^+ hybridomas and the

TABLE I

IL-2 Production by TCR γ/δ^+ T Cell Hybridomas

Exp.	Stimulus	[^{3}H]TdR incorporation*					
		BW5147	T245	1C6.F11	1C6.H10	2G8.E8	2B4
		cpm					
I	Media	2,779	1,687	1,141	1,164	1,171	1,187
	Con A, 10 μg/ml	2,389	2,632	89,664	70,858	1,171	44,746
	145-2C11, 1/10	1,865	1,973	41,062	31,080	1,154	95,520
	145-2C11, 1/100	1,419	1,860	31,914	16,022	707	46,799
II	Media	706	ND	561	439	417	ND
	Con A, 10 μg/ml	1,355		31,735	19,861	899	
	145-2C11, 1/10	834		13,539	7,051	759	
	145-2C11, 1/100	484		10,611	4,421	341	

* 10^5 T cells were cocultured for 2 d with 10^4 LS B lymphoma cells (Exp. I) or 10^5 T-depleted C3H spleen cells (Exp. II) in the presence of Con A or 145-2C11 supernatant in 0.2 ml of medium. The primary culture supernatants were assayed for IL-2 content by culturing 5×10^3 CTLL cells for 36 h in the presence of 25% primary culture supernatants. The degree of stimulation was measured by the incorporation of [^{3}H]thymidine into DNA during the final 18 h. Data are expressed as mean cpm of triplicate cultures.

$T3^+$, TCR α/β^+ hybridoma 2B4 produced easily detectable levels of IL-2 after Con A or anti-T3 stimulation. These results are consistent with the previously described anti-T3 stimulation of an uncloned murine thymocyte subpopulation (8) and a cloned human T cell line (5), both of which bear the TCR γ/δ.

The availability of large numbers of cloned T hybridoma cells that express the TCR γ/δ heterodimer should greatly facilitate the dissection of the role of this receptor in T lymphocyte development and function. These factor-independent lines should also prove to be useful in obtaining sufficient quantities of the TCR δ chain for further biochemical and molecular studies, as well as for the production of mAbs and heteroantisera. Further comparative studies of the makeup of the T3 complex in TCR α/β lines and TCR γ/δ lines may also prove to be of interest. Because we have no evidence for TCR α/β on the TCR γ/δ T cell hybridoma, our studies strongly suggest that the TCR γ/δ–T3 complex can function in a manner identical to that of the TCR α/β–T3 complex in signal transduction for IL-2 production in response to Con A and anti-T3. Further studies of the responsiveness of these cloned hybridoma lines to a wide variety of soluble as well as cell associated stimuli are in progress.

Summary

We have produced a T cell hybridoma line by fusion of an IL-2-dependent, long-term T cell receptor (TCR) γ/δ^+ Thy-1^+, bone marrow–derived, dendritic epidermal cell line to the BW5147 tumor line. The resultant hybridoma was rapidly growing, lymphokine independent, and expressed T3 in association with the TCR γ/δ heterodimer. Several subclones of the hybridoma line produced easily detectable levels of IL-2 after stimulation by anti-T3 or Con A. The availability of these cloned cell lines should greatly facilitate further functional, biochemical, and molecular studies of the TCR δ chain.

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