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The T-cell receptor γ chain–CD3 complex: Implication in the cytotoxic activity of a CD3⁺ CD4⁻ CD8⁻ human natural killer clone

(T-cell receptor γ homodimers/CD3-δ glycosylation pattern)

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ABSTRACT **A subset of human T cells has recently been described. These cells express the CD3 complex but they do not carry the classical T-cell receptor (TCR)-α/-β heterodimer on their surface (WT31⁻ CD3⁺). Instead, they express a TCR-γ chain associated with another type of polypeptide termed TCR-δ. We report here that a T-cell clone with natural killer (NK)-like activity, WM-14, had a disulfide bridged TCR-γ homodimer associated with CD3 on its surface. The TCR-γ chains of WM-14 cells were present in three different glycosylation forms of 43, 40, and 38 kDa, but they appeared to contain the same polypeptide backbone. Since cytotoxicity by WM-14 could be inhibited by anti-CD3 antibodies, we concluded that the TCR-γ–CD3 complex was involved in the NK-like unrestricted killer activity. Although normal CD3-γ, CD3-δ, and CD3-ε chains were present in this clone, the association with the TCR-γ homodimer may be the cause of a complete processing of the N-linked oligosaccharides attached to the CD3-δ chain.**

The classical T-cell receptor (TCR) for antigen (α/β heterodimer) is intimately associated with the CD3 proteins CD3-γ, -δ, -ε, and -ζ (1–6). Compelling evidence exists that if one of the polypeptide chains encoding the TCR–CD3 complex is absent, the whole complex cannot be expressed on the surface of T lymphocytes (4, 7). However, several groups have recently described a subpopulation of human thymus-derived lymphocytes that do not express the classical TCR-α/-β heterodimers as judged by RNA blot analysis, indirect immunofluorescence with the monoclonal antibody WT31, and immunoprecipitations with other anti-TCR reagents (8–11). These WT31⁻ CD3⁺ cells are phenotypically CD4⁻ CD8⁻ (11) and no function has yet been ascribed to them. The CD3⁺ WT31⁻ CD4⁻ CD8⁻ subpopulations of human thymocytes or peripheral blood lymphocytes (PBL) express a CD3-associated heterodimer of a TCR-γ chain noncovalently linked to a TCR-δ chain (8) or a single CD3-associated TCR-γ chain (12).

Here we report the characterization of a human T-cell clone (CD3⁺ WT31⁻ CD4⁻ CD8⁻) with natural killer (NK)-like unrestricted lytic capacity. This clone, WM-14, expresses a CD3–TCR-γ complex on its surface, which is involved in antigen recognition and/or cytolysis events.

MATERIALS AND METHODS

T-Cell Clones and Cell Lines. For the generation of T-cell clones, human lymphocytes were isolated from umbilical cord blood and were depleted of WT31⁺ cells by rosetting techniques (13). The WT31⁻ cells collected from the interface (10^6 cells per ml) after Ficoll/Hypaque density gradient centrifugation were stimulated with 10^5 irradiated (5000 rads; 1 rad = 0.01 Gy) JY cells per ml in Yssel's medium (14). Six days later, the cells were cloned by limiting dilution as described (15). Clones WM-37 and WM-14 were isolated and selected based on their killing capacity in ^{51}Cr release assays with JY cells as targets. A T-cell clone, HY-133, was derived from PBL of a normal donor. Six days after initial stimulation with JY cells, responder cells were cloned by limiting dilution. Responder lymphocyte clones were expanded by weekly stimulation with feeder cells as described (15).

Epstein–Barr virus-transformed B cells, JY, normal PBL, and the Daudi cell line were cultured in Yssel's medium. The human T-cell leukemic lines Jurkat and HPB-ALL were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum.

Monoclonal Antibodies and Antisera. The monoclonal antibody WT-31, which recognizes an epitope on the framework of the T-cell receptor α/β heterodimer (16, 17), was a gift of W. Tax (Nijmegen, The Netherlands). Antibodies SPV-T3b (anti-CD3), SPV-T8 (anti-CD8), SPV-L7 (anti-LFA-1), and SPV-L14 (anti-CD6) have been described (18, 19). Anti-Leu-4 monoclonal antibody (anti-CD3) was a generous gift of Robert Evans (Memorial Sloan–Kettering Institute). The βF1 monoclonal antibody (anti-TCR β-chain) was a generous gift of Michael Brenner (Dana–Farber Cancer Institute) (8). A rabbit serum specific for the human TCR-γ chain has been described (8) and was a generous gift of Siew L. Ang and John G. Seidman (Department of Genetics, Harvard Medical School). A polyclonal rabbit antiserum, H36, directed at the TCR α-chain was a generous gift of Oreste Acuto (Dana–Farber Cancer Institute).

OKT4A was purchased from Ortho Diagnostics, NKH-1 was from Coulter, and Leu-11 was from Becton Dickinson.

Flow Cytometry. The cell-surface phenotype of the human T-cell clones was analyzed by indirect immunofluorescence. T cells were incubated with murine monoclonal antibodies for 30 min at 4°C, washed, and then incubated with fluorescein isothiocyanate-labeled $F(ab)_2$ fragments of a goat anti-mouse IgG antibody (Bioart, Meudon, France) for 30 min. The samples were analyzed on a FACS IV (Becton Dickinson).

^{51}Cr Release Assays. The cytotoxic activity was determined using ^{51}Cr release assay as described (15).

RNA Blot Analysis. Total RNA was obtained by the guanidine hydrochloride method (20) and separated in a 1% agarose gel, transferred to Biodyne filters (ICN), and probed as described (21). cDNA probes were labeled with ^{32}P by the random hexadeoxyribonucleotide priming method (22). Full-length cDNA inserts encoding human TCR-β, YT35, and

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Abbreviations: TCR, T-cell receptor; NK, natural killer; PBL, peripheral blood lymphocytes; NP-40, Nonidet P-40; endo F and H, endo-β-*N*-acetylglycosaminidases F and H.

human TCR-α, pY14, polypeptide chains were obtained from Tak Mak (Ontario Cancer Institute) (23, 24). To detect TCR-γ chain mRNAs, a complete cDNA encoding HPB-MLT TCR-γ was used (25). This probe was a kindly gift of Siew L. Ang and John G. Seidman.

Radiolabeling, Immunoprecipitation, and Electrophoresis. Cells (15×10^6) of each clone were radioiodinated by the lactoperoxidase method as described (3). Labeled cells were lysed in immunoprecipitation buffers containing either 1% digitonin or 1% Nonidet P-40 (NP-40) prepared as described (3). Immunoprecipitation was performed using preformed immune complexes. Briefly, after lysing cells in immunoprecipitation buffer, lysates were centrifuged first for 15 min at 13,000 $\times$ *g* in an Eppendorf centrifuge and then in a Beckman airfuge for 30 min at 100,000 $\times$ *g*. Supernatants were precleared overnight with 10 μl of *Staphylococcus aureus* (Pansorbin, Calbiochem) and then three times for 1 hr at 4°C with preformed complexes of either nonimmune mouse or rabbit sera and rabbit anti-mouse IgG or goat anti-mouse IgG antisera (Cooper Biomedical, Malvern, PA). After the preclearing steps, supernatants were incubated for 2–4 hr with preformed complexes of the specific antibodies.

For immunoprecipitation with the rabbit anti-peptide antibody specific for human TCR-γ constant regions, the protocol varied slightly. After the preclearing steps, samples were heated for 5 min at 55°C in the presence of 1% $NaDodSO_4$ and 1 mM dithiothreitol. After cooling the samples and adding 2 mM iodoacetamide, samples were diluted 1:4 with NP-40-containing buffer and incubated for 4 hr with preformed complexes of the anti-TCR-γ antibody.

Immunoprecipitates were washed five times with the immunoprecipitation buffer. Electrophoresis was performed by the $NaDodSO_4$/PAGE standard method in 12.5% polyacrylamide gels unless otherwise indicated.

Elution of Proteins and Immunoprecipitation of the Eluted Proteins. After the proteins of interest were localized by autoradiography, they were eluted by electroelution as described (26) in 0.05 M ammonium bicarbonate buffer containing 0.1% $NaDodSO_4$ and protease inhibitors. One hundred micrograms of whale apomyoglobin (Beckman) was added per sample. After an overnight elution, samples were incubated with either a nonimmune serum or a specific antibody absorbed to protein A-Sepharose beads (Pharmacia). After incubating 2–4 hr at 4°C, beads were washed three times with NP-40 immunoprecipitation buffer and prepared for electrophoresis.

Endo-β-*N*-Acetylglycosaminidases F (endo F) and H (endo H) Treatments. Proteins eluted by the method described above were precipitated by incubation for 1 hr at 4°C with 10% trichloroacetic acid. Samples were centrifuged at 13,000 $\times$ *g* for 15 min and pellets were washed three times with acetone and dried. For endo H treatments, samples were boiled with 1.2-fold quantities of $NaDodSO_4$ and then diluted 1:4 with 50 mM sodium citrate buffer (pH 5.5). Samples were then incubated with endo H (0.04 unit/ml) (Genzyme, Norwalk, CT) for 6 hr, followed by precipitation with trichloroacetic acid, washing with acetone, drying, and electrophoresis as described above.

For endo F treatment, the procedure was similar, but proteins after the first trichloroacetic acid precipitation were boiled in 30 μl of 100 mM Tris·HCl buffer (pH 6.8) containing 1% 2-mercaptoethanol, 0.5% $NaDodSO_4$, and 10 mM EDTA. Afterwards, protease inhibitors and 1% NP-40 were added. Samples were incubated with endo F for 4 hr and trichloroacetic acid-precipitated as described above. Endo F was a generous gift from James Kaufman (Basel Institute of Immunology).

RESULTS

The T-cell clone WM-14 was isolated from a T-cell culture from which the $WT31^+$ cells were removed by rosetting. The remaining $WT31^-$ cells were subsequently stimulated with JY cells. As shown in Table 1, this clone expresses CD3 but does not react with WT31. In addition, WM-14 reacted with the antibody NKH-1, which defines human NK cells. A second clone, HY-133, which was isolated from normal PBL and stimulated with JY cells, also reacted with NKH-1. In contrast to WM-14, HY-133 reacted strongly with both WT31 and anti-CD3 antibodies. Furthermore, clone HY-133 reacted with the anti-CD8 antibody SPV-T8, but not with OKT-4A, whereas WM-14 was negative for both CD8 and CD4 (Table 1). As a control, we included clone WM-37, which was $CD3^+$ $WT\text{-}31^+$ $CD8^-$ $CD4^+$. WM-37 also expressed CD6, which was not found on WM-14 or HY-133. The latter clones represent a minor $CD3^+$ T-cell population, since we have recently found that only 2–3% of the $CD3^+$ T cells failed to express CD6 (19).

Both WM-14 and HY-133 displayed NK-like activities (Table 2), since they lyse K-562 cells as well as JY cells. In addition, WM-14 was found to lyse Epstein–Barr virus-transformed B cells, but not normal lymphocytes or phytohemagglutinin blasts from the same donor (Table 2). These results indicate that WM-14 and HY-133 represent a minor subset of $CD3^+$ NK clones.

Table 2 also shows that the anti-CD3 antibody SPV-T3b inhibited the cytotoxic activity of WM-14 and HY-133 against JY cells, whereas WT31 inhibited only the activity of HY-133. These results indicate that the CD3 complex mediated the lytic activity by these clones. To investigate the nature of the putative antigen receptors that are associated with the CD3 proteins on the surface of WM-14 and HY-133, further experiments were done.

RNA Blot Analysis. RNA blot analysis of WM-14 RNA showed that this clone has transcripts for the TCR-γ gene (Fig. 1). This clone had, however, no detectable TCR-α mRNA and only the truncated form [1.0 kilobase (kb)] of TCR-β mRNA. In comparison, the human leukemic T-cell line HPB-ALL and the $WT31^+$ clone HY-133 each had a 1.6-kb TCR-α mRNA and a full-length 1.3-kb TCR-β mRNA. Like WM-14, HPB-ALL cells had a smaller quantity of TCR-γ transcripts, whereas HY-133 did not express any detectable TCR-γ-chain mRNA.

Immunoprecipitation of CD3-Associated Molecules. To study the association between the TCR proteins and the CD3 polypeptides of WM-14, surface radioiodinated cells were lysed in the presence of the mild detergent digitonin as described (3). As shown in Fig. 2*A* (lane a) under nonreducing conditions, a 78-kDa protein coprecipitated with CD3 proteins from WM-14. In contrast, an 85-kDa protein, which migrated with approximately the same mobility as the TCR from Jurkat cells, was precipitated from clone HY-133. To analyze the existence of interchain disulfide bridges, the CD3-associated proteins were eluted and separated by $NaDodSO_4$/PAGE under reducing conditions. The 78-kDa protein of WM-14 cells (protein 1, Fig. 2*A*) migrated as three protein bands after reduction—namely, two main proteins of 43 and 40 kDa and variable amounts of a 38-kDa protein (Fig. 2*B*, lane a). The 85-kDa band of HY-133 (protein 2, Fig. 2*A*)

Table 1. Indirect immunofluorescence analysis of WM-14, WM-37, and HY133 T-cell clones

Clone	TCR-α/-β (WT31)	CD3 (SPV-T3b)	CD8 (SPV-T8)	CD4 (OKT-4A)	NKH-1	CD6 (SPV-L14)
WM-14	−	+	−	−	+	−
WM-37	+	+	−	+	−	+
HY-133	+	+	+	−	+	−

Table 2. Cytotoxic activity of WM-14 and HY-133 against K-562 and JY cells: Effect of monoclonal antibodies on the cytotoxic activity of these clones

Target cells	Monoclonal antibody added†	% ^{51}Cr release* Clone WM-14	Clone HY-133
K-562	—	42	51
Daudi	—	31	ND
EBV B cells	—	25	ND
PBL	—	0	ND
PHA blasts‡	—	0	ND
JY	—	52	30
JY	SPV-T3b (CD3)	7	7
JY	WT31 (TCR-α/-β)	53	20
JY	SPV-L14 (CD6)	54	31

ND, not determined; EBV, Epstein–Barr virus; PHA, phytohemagglutinin.
*An effector-to-target ratio of 2:1 was used.
†The monoclonal antibodies were used in a dilution of 1:100 of ascites.
‡Phytohemagglutinin blasts were obtained by incubating 10^6 PBL per ml with 0.1 mg of phytohemagglutinin per ml for 4 days.

was composed of two major proteins (43 and 46 kDa) and a minor protein of 40 kDa (Fig. 2*B*, lane b). In the case of the T-cell receptor from Jurkat cells, two α-chains (47 and 45 kDa) and a β-chain (40 kDa) were found after reduction (Fig. 2*B*, lane c).

The elution and reduction experiments were in agreement with direct analysis by NaDodSO$_4$/PAGE of the CD3 complex under reducing conditions. Three well-resolved CD3-associated proteins of 43, 40, and 38 kDa (Fig. 2*C*, lane b) were isolated from WM-14 cells. In comparison, the WT31$^+$ clone WM-37, which was used as a control, had only two CD3-associated proteins of 43 and 39 kDa.

Since clone WM-14 contained TCR-γ mRNA but no full-length TCR-α/-β transcripts, we concluded that the disulfide-linked dimer of 78 kDa was likely to contain a TCR-γ protein associated with CD3.

Immunoprecipitation of WM-14 Membrane Proteins with an Anti-TCR-γ Antibody. To examine whether TCR-γ protein was indeed expressed on the surface of WM-14 cells, immunoprecipitations were carried out with an antibody raised against a peptide common to the TCR-γ constant regions. The anti-TCR-γ reagent precipitated a 78-kDa protein (Fig. 3*A*, lane g) that had the same mobility as the CD3-associated protein (Fig. 3*A*, lane a). An antibody, βF1, reacting with TCR-β-chains detected the β-chain (lane f) of Jurkat cells but did not precipitate any specific protein from WM-14 cells

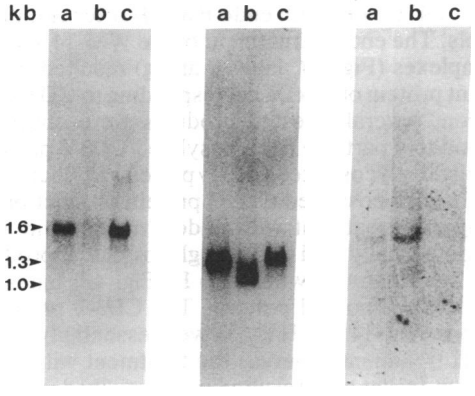

FIG. 1. RNA blot analysis of TCR gene transcripts. Total RNA (18 μg) from HPB-ALL (lanes a), WM-14 (lanes b), and HY-133 (lanes c) cells were loaded. After blotting, the filter was sequentially hybridized with TCR-α (*Left*), -β (*Center*), and -γ (*Right*) probes.

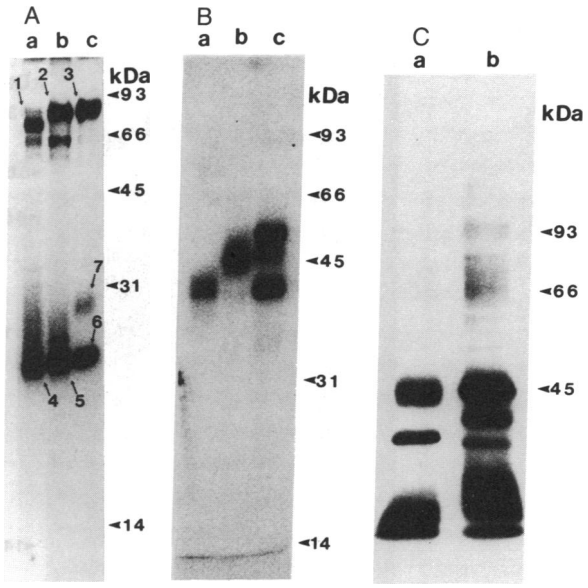

FIG. 2. Immunoprecipitation of WM-14 and HY-133 TCR–CD3 complexes. (*A*) Electrophoresis under nonreducing conditions of anti-Leu-4 immunoprecipitates from WM-14 cells (lane a), HY-133 cells (lane b), and Jurkat cells (lane c). The immunoprecipitation was done in digitonin-containing buffer. (*B*) Electrophoresis under reducing conditions in 10% polyacrylamide gel of proteins 1, 2, and 3 eluted from gel shown in *A*. Reduction products of protein 1 from WM-14 cells (lane a), of protein 2 from HY-133 cells (lane b), and of protein 3 from Jurkat cells (lane c) are shown. (*C*) Electrophoresis under reducing conditions in 7% polyacrylamide gel of anti-Leu-4 immunoprecipitates from the WT31$^+$ clone 37 (lane a) and the WM-14 clone (lane b).

(lane d). Unlike anti-CD3, the anti-TCR-β and anti-TCR-γ antibodies did not precipitate the complete TCR–CD3 complex. This discrepancy is due to the use of NP-40 instead of digitonin for solubilization. Both the CD3-associated 78-kDa protein (protein 1, Fig. 3*A*) and the 78-kDa protein precipitated by the anti-TCR-γ antibody (protein 2) migrated as three protein bands of 43, 40, and 38 kDa under reducing conditions (Fig. 3*B*). In fact, proteins 1 and 2 of Fig. 3*A* are probably identical.

The 43-, 40-, and 38-kDa CD3-associated proteins were further analyzed by elution and reprecipitation (proteins 5a, 5b, and 6 of Fig. 3*B*) with the anti-TCR-γ antibody, which recognized all three proteins (Fig. 3*C*). An irrelevant protein, used as a negative control, was not immunoprecipitated at all by the anti-TCR-γ antibody (not shown). Proteins 3 and 4 of Fig. 3*A* were also reprecipitated by the anti-TCR-γ antibody (not shown). This suggests strongly that proteins 3 and 4 are partially reduced forms of the 78-kDa protein resulting from the use of 1 mM dithiothreitol in the immunoprecipitation protocol.

In similar experiments, none of the 43-, 40-, and 38-kDa proteins of anti-Leu-4 immunoprecipitates from WM-14 cells was reprecipitated by the anti-TCR-β antibody, βF1, or by H36, an antipeptide rabbit antiserum specific for human TCR-α constant region (not shown). By contrast, the 43- and 40-kDa proteins of HY-133 (see Fig. 2*B*, lane b) were reprecipitated by the anti-TCR-β antibody and the 46-kDa protein by the anti-TCR-α antibody (not shown).

Our interpretation of these results is that the WM-14 clone has a receptor composed of two or three TCR-γ products associated with the CD3 complex, whereas the HY-133 clone has a TCR-α/-β heterodimer. However, it cannot be excluded that a TCR-δ-chain is among these proteins.

Endo F Treatment of WM-14 TCR Proteins. To study the polypeptide backbone of the TCR proteins, these proteins

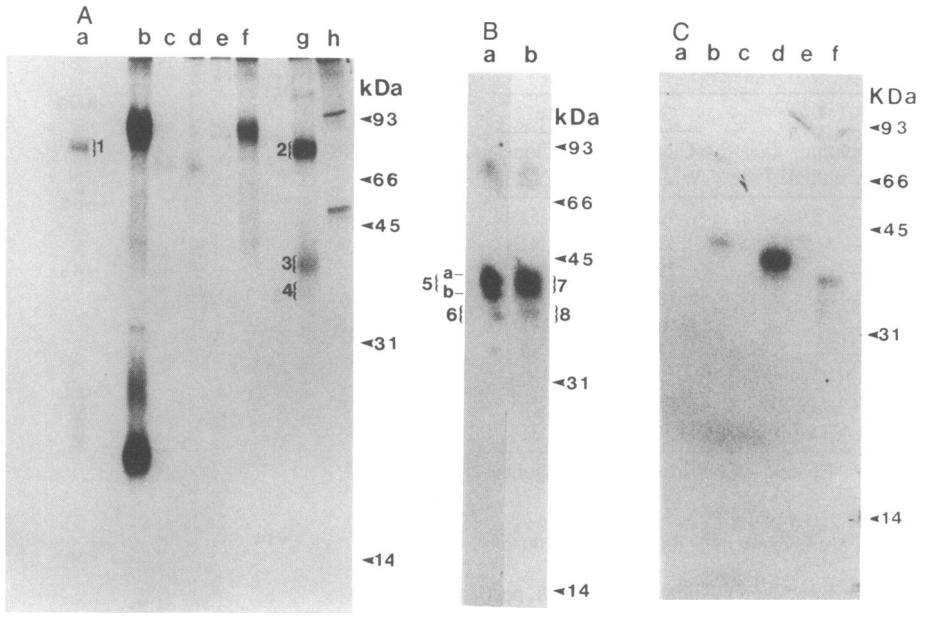

FIG. 3. Analysis of CD3-associated molecules in clone WM-14. (*A*) Electrophoresis under nonreducing conditions of anti-Leu-4 immunoprecipitates obtained in the presence of digitonin from WM-14 cells (lane a) or Jurkat cells (lane b). Immunoprecipitates prepared in 1% NP-40 were done with nonimmune serum (lanes c and e), with βF1 antibody (lanes d and f), or with an anti-TCR-γ antibody (lanes g and h). In these cases, lysates were obtained from WM-14 cells (lanes c, d, and g) or from Jurkat cells (lanes e, f, and h). (*B*) Reduction products of proteins 1 (lane a) and 2 (lane b) eluted from *A*. (*C*) Reprecipitation with anti-TCR-γ antiserum of proteins 5a, 5b, and 6 from *B*. After elution, proteins 5a (lanes a and b), 5b (lanes c and d), and 6 (lanes e and f) were reprecipitated with nonimmune rabbit serum (lanes a, c, and e) or the anti-TCR-γ antiserum (lanes b, d, and f).

were eluted from the gel shown in Fig. 3*B* (proteins 7 and 8) and treated with endo F. As shown in Fig. 4*A* (lane a) some of the 43- and 40-kDa proteins remained undigested (bands 1 and 2) but part was digested to two products of 38 and 35 kDa (bands 3 and 4). The treatment with endo F of the 38-kDa protein resulted in some nondigested protein and a digested product of 35 kDa (Fig. 4*A*, lane b). Since band 4 is in both lanes a and b, it is likely that the 35-kDa protein is the final digestion product of the 43-, 40-, and 38-kDa proteins. This would imply that the 40-kDa and the 38-kDa proteins are partially glycosylated forms, and the 43-kDa protein is the fully glycosylated form of the same protein. Taken together these data support the notion that the WM-14 clone has a TCR-γ homodimer associated with CD3 proteins, and this receptor is expressed on the membrane in three differently glycosylated forms. The possibility that a TCR-δ chain is hidden among these bands is, however, not excluded.

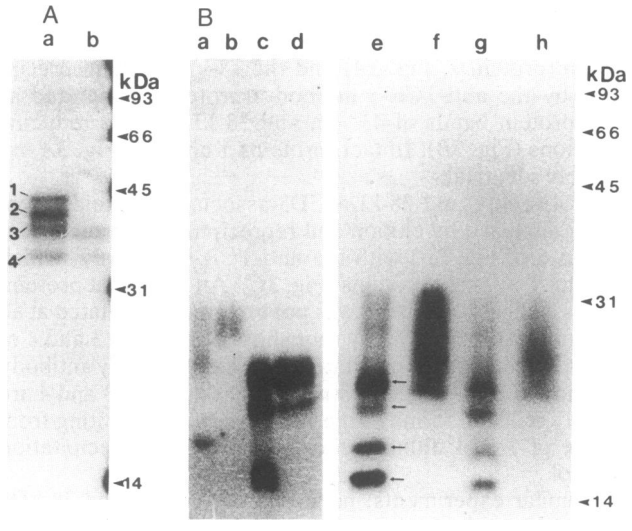

FIG. 4. Deglycosylation of the CD3 and CD3-associated complex proteins from clones WM-14, HY-133, and Jurkat cells. (*A*) Endo F treatment of protein 7 eluted from gel in Fig. 3*B* (lane a) and of protein 8 (lane b) eluted from the same gel. (*B*) Endo F and H treatments of proteins 4, 5, 6, and 7 of Fig. 2*A*. The CD3-γ protein of Jurkat cells (protein 7 in Fig. 2*A*) was digested either with endo F (lane a) or endo H (lane b). The CD3-δ and CD3-ε proteins of Jurkat cells (protein 6 in Fig. 2*A*) were eluted together and digested either with endo F (lane c) or endo H (lane d). The whole CD3 complex from WM-14 cells (protein 4 in Fig. 2*A*) was isolated and treated with endo F (lane e) or endo H (lane f). The whole CD3 complex from HY-133 clones (protein 5 in Fig. 2*A*) was isolated and treated with endo F (lane g) or endo H (lane h). Arrows indicate position of proteins cited in the text.

Endo F and H Treatments of CD3 Proteins. Immunoprecipitates prepared with anti-CD3 reagents from WM-14 and HY-133 clones (Figs. 2*A* and 3*A*) contained unusually diffuse CD3 bands. To demonstrate the presence of each of the CD3 complex chains, the whole 20- to 28-kDa CD3 region from the gel in Fig. 2*A* (proteins 4 and 5) was eluted and treated with endo F and endo H. The 20-kDa proteins (CD3-δ and CD3-ε) and the 28-kDa protein (CD3-γ) from Jurkat cells (proteins 6 and 7, Fig. 2*A*) treated with endo F and H after elution served as controls. The endo F treatment of the WM-14 and HY-133 CD3 complexes (Fig. 4*B*, lanes e and g) resulted in an endo F-resistant protein of 20 kDa corresponding to CD3-ε protein. In addition, several digested products were observed that correspond to a partially deglycosylated CD3-δ protein of 18 kDa, a fully deglycosylated CD3-γ protein product of 16 kDa, and a fully deglycosylated CD3-δ protein product of 15 kDa, indicating that treatment with endo F resulted in a normal pattern of partially and fully deglycosylated products. In contrast, the treatment with endo H (Fig. 4*B*, lanes f and h) resulted in an abnormal pattern. The CD3-δ proteins from both clones WM-14 and HY-133 were essentially insensitive to endo H treatment, whereas the treatment with endo H of CD3-δ from Jurkat cells resulted in a partially deglycosylated form of 18 kDa (see also ref. 2).

Taken together, these data indicate that the CD3-γ, -δ, and -ε chains were present on the surface of WM-14 and HY-133 cells. CD3-δ was found on the surface of WM-14 and HY-133

clones with two endo H-resistant N-linked complex-type oligosaccharides.

DISCUSSION

This report describes two $CD3^+$ NK cell clones, WM-14 and HY-133, which both express NKH-1 but differ with respect to the expression of the WT31 determinant and the CD8 antigen. These clones displayed NK-like cytotoxic activity and, since this activity is inhibited by an anti-CD3 monoclonal antibody, CD3 must be involved in the lytic activity of both clones. WM-14 was found to be negative for WT31, CD8, and CD4. Therefore, WM-14 may represent a subset of $CD3^+$ $CD4^-$ $CD8^-$ cells described earlier (1, 27). Here we show that WM-14 expresses the TCR-γ chain in association with the CD3 proteins, whereas the $WT31^+$ T-cell clone HY-133 expresses the α/β heterodimer. In this respect, WM-14 resembles the $CD4^-$ $CD8^-$ thymocyte clone described by Bank *et al.*, which also expressed TCR-γ in association with CD3 (9). In contrast, no function was ascribed to this latter clone. Since it has been shown that the $CD4^-$ $CD8^-$ thymocytes can become cytotoxic after culture in interleukin 2 (28), it is not unreasonable to assume that $CD4^-$ $CD8^-$ thymocytes can be compared with $CD4^-$ $CD8^-$ T lymphocytes. However, the WM-14 clone is different from the recently described $WT31^-$ clones isolated from fetal blood, since the latter expressed CD8 (10).

Although in most of the NK cells (phenotypically $CD3^-$ $CD16^+$) there is not a rearranged TCR-γ gene (29), it seems that in the WM-14 clone the CD3-associated TCR-γ products are functionally implicated in the killing of targets, since anti-CD3 antibodies inhibited killing.

WM-14 cells seem to express on the surface a TCR-γ homodimer composed of a 35-kDa polypeptide backbone, which can be present in differently glycosylated forms with molecular masses between 38 and 43 kDa. These TCR-γ proteins are disulfide bound, and for this reason the WM-14 clone resembles the case of the NK clones F6A4 and F6C7 (30) in which a disulfide-bound heterodimer of unknown nature is associated with the CD3 complex. Previously, a TCR-γ protein of 55 kDa had been found associated with CD3 in PEER cells (12) and in a peripheral T-lymphocyte subset of immunodeficient patients (8). In the latter case, the 55-kDa protein was noncovalently linked to a 40-kDa protein called δ. We cannot exclude totally the existence of such a protein in the WM-14 clone, but the deglycosylation experiment (Fig. 4*A*) suggested that such a species is not present.

Unlike the CD3-δ chains of most T lymphocytes, the CD3-δ from WM-14 contained mainly complex N-linked oligosaccharides that were insensitive to digestion with endo H. The maturation of the glycan moieties on the CD3-δ protein from clone WM-14 could be dependent on the mode of association between the TCR-γ polypeptide chains and CD3. A similar interdependence of oligosaccharide processing on protein–protein association has been reported for the HLA-A/-B heavy chains and β_2-microglobulin (31). On the other hand, clone HY-133 expressing a TCR-α/β heterodimer also expressed a CD3-δ containing only complex N-linked oligosaccharides; hence, the glycosylation pattern of the CD3 proteins may depend on the growth state of the cells. It remains to be determined why cells expressing TCR-γ should have different growth properties from the other resting T lymphocytes in certain immunodeficiency patients (8).

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