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Immunology

Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity

(chimeric genes/antibody variable region)

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ABSTRACT **To design and direct at will the specificity of T cells in a non-major histocompatibility complex (MHC)-restricted manner, we have generated and expressed chimeric T-cell receptor (TcR) genes composed of the TcR constant (C) domains fused to the antibody's variable (V) domains. Genomic expression vectors have been constructed containing the rearranged gene segments coding for the V region domains of the heavy (V_H) and light (V_L) chains of an anti-2,4,6-trinitrophenyl (TNP) antibody (SP6) spliced to either one of the C-region gene segments of the α or β TcR chains. Following transfection into a cytotoxic T-cell hybridoma, expression of a functional TcR was detected. The chimeric TcR exhibited the idiotope of the Sp6 anti-TNP antibody and endowed the T cells with a non-MHC-restricted response to the hapten TNP. The transfectants specifically killed and produced interleukin 2 in response to TNP-bearing target cells across strain and species barriers. Moreover, such transfectants responded to immobilized TNP-protein conjugates, bypassing the need for cellular processing and presentation. In the particular system employed, both the TNP-binding site and the Sp6 idiotope reside almost exclusively in the V_H chain region. Hence, introduction into T cells of TcR genes containing only the V_HSp6 fused to either the Cα or Cβ was sufficient for the expression of a functional surface receptor. Apparently, the V_HCα or V_HCβ chimeric chains can pair with the endogenous β or α chains of the recipient T cell to form a functional $\alpha\beta$ heterodimeric receptor. Thus, this chimeric receptor provides the T cell with an antibody-like specificity and is able to effectively transmit the signal for T-cell activation and execution of its effector function.**

Antigen recognition by T-cell receptors (TcRs) differs from that of antibodies in that antibodies interact with antigens in their native state with relatively high affinity, whereas TcRs recognize fragmented antigens bound to cell surface class I and class II major histocompatibility complex (MHC) molecules (1). The differences in specificity of TcRs versus antibodies are not, however, evident from differences in their molecular structure. In fact, both molecules consist of two disulfide-linked polypeptide chains referred to as α and β for the TcR and heavy (H) and light (L) for antibodies, each containing constant (C) and variable (V) domains. The antigen-binding site of both antibodies and TcR is encoded by a V-region exon, formed by rearrangment of V, diversity and joining gene segments (reviewed in ref. 2). In the case of immunoglobulins, x-ray data have shown that the V regions are loosely connected to the C regions (3), and, because of their high degree of homology with immunoglobulin, it is highly likely that TcRs are constructed similarly. Therefore, from a structural standpoint, it should be possible to confer antibody specificity on T cells by removing TcR V regions and replacing them with antibody V regions. Such a "chimeric TcR" (cTcR) would contain the extracellular C region, the transmembrane segment, and the cytoplasmic domains of normal TcRs and should therefore be able to function normally to induce T-cell proliferation, interleukin production, and target cell lysis.

Spontaneous transcription of an aberrantly joined IgV_H gene and a TcR JαCα gene resulting from site-specific chromosome 14 inversion in human T-cell tumors was reported (4–6); however, no protein product was detected. Chimeric fusion proteins have also been produced in myelomas by the introduction of the TcR C exons between the V_κ and C_κ exons (7). More recent reports have shown that a chimeric protein containing the TcR V_α domain and the immunoglobulin C domain can be synthesized in myeloma cells. This protein associates with normal L chains to form a secreted tetramer (8). Attempts to assemble and secrete similar chimeric protein containing the $V_\alpha C_\mu$ and $V_\beta C_\kappa$ have not been successful (9).

The studies described above all reported the construction of nonfunctional chimeric Ig-TcR proteins. In this paper we describe the construction and functional expression in T cells of chimeric TcR genes made by recombining the immunoglobulin V_H and V_L rearranged gene segments to the C-region exons of the TcR α and β chains. The resulting cTcR is expressed on the surface of cytotoxic T lymphocytes, recognizes antigen in a non-MHC-restricted manner, and effectively transmits the transmembrane signal for T-cell activation.

MATERIALS AND METHODS

Construction of Chimeric Genes: Isolation of V_L and V_H Gene Segments. Genomic clones containing the rearranged VJ_κ ($T_\kappa 1$ plasmid) and VDJ_H (pR-Sp6 plasmid) derived from the IgM ($\mu\kappa$) anti-2,4,6-trinitrophenyl (TNP)-producing Sp6 hybridoma (10–11) were kindly provided by G. Köhler (Max-Planck-Institut für Immunbiologie). The 2.2-kilobase-(kb) *Pst* I fragment containing L-VJ_κ from $T_\kappa 1$ and the 1.7-kb *Xba* I fragment containing L-VDJ_H were isolated, and each was subcloned into pUC19.

Isolation of TcR Cα and Cβ Clones. Two genomic clones, λCα2 and λCβ11, which contained all of the C exons and a large portion of the upstream intron, were isolated from a mouse embryonic library in Charon 4A (12) using Cα and Cβ probes derived from the TT11 and 86T1 cDNA clones, respectively, kindly provided by M. M. Davis (Stanford University School of Medicine). A 9.0-kb *Bam*HI fragment containing all Cα exons plus 5′ 1.0 kb and a 6.3-kb *Bam*HI

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Abbreviations: TcR, T-cell receptor; cTcR, chimeric TcR; MHC, major histocompatibility complex; V, variable; C, constant; H, heavy; L, light; mAb, monoclonal antibody; TNP, 2,4,6-trinitrophenyl; IL-2, interleukin 2; APC, antigen-presenting cell; LTR, long terminal repeat; CTL, cytotoxic T lymphocyte; RSV, Rous sarcoma virus.

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fragment containing all of the Cβ2 exons plus 5′ 1.9 kb were each subcloned into the *Bam*HI site of pUC19.

Cloning of the Chimeric Genes into Expression Vectors. The vectors used for construction and transfection of the chimeric Ig-TcR genes were pRSV2neo and pRSV3gpt (kindly provided by DNAX). These are derivatives of pSV2neo (13) and pSV3gpt (14) vectors into which the long terminal repeat (LTR) of the Rous sarcoma virus (RSV) was introduced. To construct the V_L-containing chimeric vectors, we isolated from the Tκ1 plasmid a 0.9-kb *Bgl* II–*Bam*HI fragment by partial *Bgl* II digestion followed by *Bam*HI digestion. This fragment, containing the complete L-VJ_κ segment, was inserted into the *Bam*HI site of pRSV3gpt to produce pRSV3V_L, in which L-VJ_κ is in the correct transcription orientation with regard to the RSV-derived LTR. Into the 3′ *Bam*HI of the insert, the 9.0-kb *Bam*HI containing Cα or the 6.3-kb *Bam*HI fragment containing Cβ2 was introduced to generate pRSV3V_LCα or pRSV3V_LCβ, respectively (Fig. 1). The V_H-containing chimeric vectors were constructed by introducing in the correct orientation into the *Bam*HI site of pRSV2neo a 1.6-kb *Bcl* I–*Bam*HI fragment containing the L-VDJ_H of Sp6. The remaining 3′ *Bam*HI site was then used to insert the *Bam*HI Cα and Cβ fragments described above to produce pRSV2V_HCα and pRSV2V_HCβ (Fig. 1), respectively. For both V_L and V_H constructions, we used naturally occurring restriction sites complementary to the *Bam*HI site (*Bgl* II in V_L and *Bcl* I in V_H), which reside 25 and 45 base pairs, respectively, upstream from the starting ATG codon of both leader peptide exons. No other ATG is present between the LTR promoter and the starting codons in either case. This ensures that the original starting methionine will be conserved and that no transcriptional control elements of the immunoglobulin genes will be included.

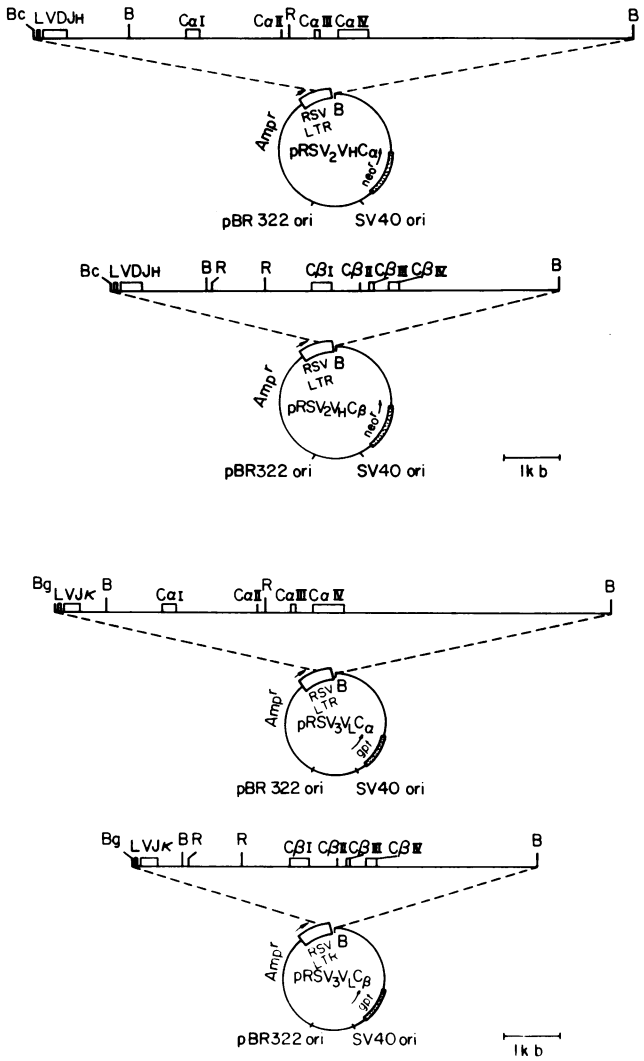

FIG. 1. Scheme of the chimeric antibody/TcR genomic constructs inserted into the *Bam*HI (B) site of the pRSV expression vectors. The boxes along the linear insert represent (from left to right) the exons coding for the leader (L), rearranged V region of the H chain (VDJ_H) or κ L chain (VJ_κ), and the four C domains of the TcR α and β chains. SV40, simian virus 40.

Transfection. MD.45, an *H-2D*^{b} allospecific cytotoxic T-cell hybridoma (15), was transfected by protoplast fusion as described by Ochi *et al.* (16). Transfectants receiving pRSV2neo- or pRSV3gpt-based vectors were selected for growth in the presence of G418 (2 mg/ml) (GIBCO) or mycophenolic acid (1 μg/ml) (GIBCO), hypoxanthine (15 μg/ml) (Sigma), and xanthine (200 μg/ml) (Sigma), respectively.

RNA Analysis. Cytoplasmic RNA was prepared from transfected cells (17). Northern blots were performed by standard techniques and probed with the following probes labeled by nick-translation: Cα, 0.55-kb *Nco* I fragment of TT11 plasmid; Cβ, 0.3-kb *Xho* I–*Nco* I fragment of 86T1 plasmid; V_H, 1.7-kb *Xba* I fragment of pR-Sp6 (11); V_L, 0.9-kb *Bgl* II fragment of T_κ1 (10).

Immunoblotting. Pellets containing 2×10^7 cells were lysed in 200 μl of solution containing 1% Nonidet P-40, 10 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (Sigma), 10 μg of aprotinin per ml (Sigma), and 10 units of leupeptin per ml (Sigma) in 10 mM Tris, pH 8.0/0.15 M NaCl. After 30 min at 0°C and centrifugation at 12,000 × *g*, 50 μl of each supernatant was boiled for 2 min in sample buffer containing 10 mM iodoacetic acid. $NaDodSO_4$/PAGE through 10% gels was performed by the method of Laemmli (18). Separated proteins were blotted onto nitrocellulose filters (19) and allowed to react with the anti-Sp6 idiotypic monoclonal antibody (mAb) 20.5 (20) (provided by G. Köhler), followed by affinity-purified ^{125}I-labeled goat anti-mouse Fab′ antibody.

Interleukin 2 (IL-2) Secretion Assays. Transfectants were incubated with various target cells and antigens in Dulbecco's modified Eagle's medium containing 10% fetal calf serum for 18–36 hr. IL-2 secretion was assayed by using an IL-2-dependent cell line and the methyltetrazolium staining (21). One unit of IL-2 is defined as an inverse of the dilution that supported 50% growth of the cytotoxic T lymphocyte CTL-L indicator cells.

Cytotoxicity Assay. The ^{51}Cr-release assay (15) was used to evaluate the ability of the transfectants to lyse specific target cells. Target cells were modified by TNP using 10 mM 2,4,6-trinitrobenzenesulfonic acid as described (22). Killing assays were performed at different ratios of effector to ^{51}Cr-labeled target cells for 4–8 hr.

RESULTS

Construction and Expression of Chimeric TcR Genes. To produce a chimeric TcR with an antigen-binding site of a given antibody molecule, we have combined each of the rearranged L-VDJ_H and L-VJ_κ gene segments of the anti-TNP mAb Sp6 with gene segments encoding the C region of either one of the α or β TcR chains. In the final expression vector, transcription is driven by the RSV LTR inserted 5′ to the immunoglobulin leader exon, next to the starting ATG codons. Fig. 1 depicts schematically the different chimeric genes and the expression vectors used. Because we could not predict whether a certain combination of V_H or V_L combined with either Cα or Cβ will result in better pairing with the complementary chain, we constructed all four combinations of chimeric genes—V_HCα, V_HCβ, V_LCα, and V_LCβ. Plasmids were constructed so that each cTcR chain was associ-

ated with a different selectable marker (*gpt* or *neo*^{r} genes) than was its complementary chain, to allow selection of transfectants coexpressing both chimeric genes. To express these chimeric genes in T cells, we transfected them into the MD.45 murine hybridoma. The MD.45 hybridoma specifically lyses *H-2D*^{b} target cells (15) and, upon stimulation with either T-cell mitogens or appropriate target cells, produces IL-2, interleukin 3, and granulocyte/macrophage colony-stimulating factor. The hybridoma subclone used in this study expresses both of the α and β TcR chains of its CTL parent and only the β chain of BW5147 (23). We first transfected into MD.45 each of the four chimeric constructs independently and analyzed the resulting transfectants for expression of chimeric transcripts. We then retransfected the positive clones with the complementary constructs.

In the first round of transfections with single chimeric genes, out of 48 wells seeded for each transfection, growth was seen in 21 of those that received $V_LC\alpha$ (termed GTA.a), 15 that received $V_LC\beta$ (termed GTA.b), 9 that received $V_HC\alpha$ (termed GTA.c), and 13 that received $V_HC\beta$ (termed GTA.d). Clones expressing high RNA levels of each series were then transfected with the complementary construct. In all, out of 3×10^7 cells transfected, 54 independent transfectants were obtained from GTA.a that received the $V_HC\beta$ construct (termed GTA.e), 13 from GTA.b that received $V_HC\alpha$ (termed GTA.f), 10 from GTA.c that received $V_LC\beta$ (termed GTA.g), and 18 from GTA.d that received $V_LC\alpha$ (termed GTA.h).

To determine which of the transfectants expressed V_H and/or V_L chimeric gene segments, we analyzed their RNA transcripts by Northern blot hybridization analysis using probes specific for the Sp6 V_H (Fig. 2*A*, upper panel) and V_L (Fig. 2*A*, lower panel) regions. As a positive control, total cytoplasmic RNA from the Sp6 hybridoma gave a strong signal corresponding to mRNA of μ H chain and κ L chain. No hybridization was observed with RNA of the untransfected cells (second lane). The transfectants that received V_L-containing chimeric genes (represented by a.2 and b.2 in Fig. 2) hybridized only with V_L probes and the transfectants that received the $V_HC\alpha$ (c.2) or $V_HC\beta$ (d.9) hybridized only with the V_H Sp6 probe. Almost all of the transfectants that received both chimeric genes (represented by clones e, f, g, and h) transcribed both RNAs into transcripts of the predicted size (1.4 kb for $V_LC\beta$ or $V_HC\beta$ and 1.7 kb for $V_LC\alpha$ or $V_HC\alpha$). The level of expression of chimeric RNA was far greater than the expression of the endogenous TcR α and β chains of the same cells (data not shown).

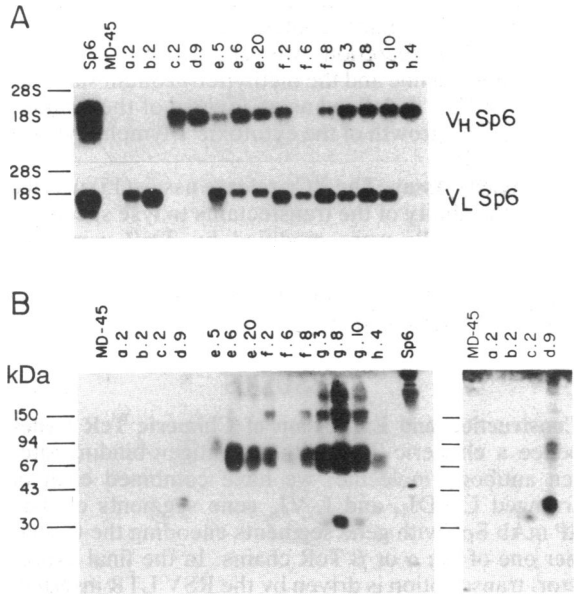

FIG. 2. Expression of the chimeric TcR chains. (*A*) Northern blot analysis of cytoplasmic RNA using ^{32}P-labeled V_HSp6 and V_LSp6 probes. (*B*) Western immunoblot analysis using anti-Sp6 idiotypic mAb and ^{125}I-labeled anti-mouse immunoglobulin. Cell lysates were separated by NaDodSO$_4$/PAGE under nonreducing conditions. The right panel represents a 4-fold longer exposure of the first five left lanes. Transfectants analyzed: a, $V_LC\alpha$; b, $V_LC\beta$; C, $V_HC\alpha$; d, $V_HC\beta$; e and h, $V_LC\alpha$ + $V_HC\beta$; f and g, $V_LC\beta$ + $V_HC\alpha$.

Expression of the chimeric polypeptide chains was analyzed in cell lysates by immunoblotting using the anti-Sp6 idiotypic mAb 20.5 (Fig. 2*B*). The idiotope recognized by this antibody is sensitive to reduction (G.G. and Z.E., unpublished data) and is expressed exclusively by the V_H of the Sp6 μ chain: the antiidiotypic mAb reacted only with transfectants that received chimeric genes containing V_H either alone (such as c.2 and d.9, shown after longer exposure of the blot, right panel of Fig. 2*B*) or together with V_L chimeric genes (e–h, Fig. 2*B*). The doubly transfected cells expressed a major broad band (apparently composed of two major bands of 85 and 90 kDa) that corresponds to heterodimeric proteins, bands of higher molecular mass of about 160 kDa, and higher molecular mass bands that most likely correspond to heterotetrameric and higher complexes of the chimeric receptor. Bands of about 38 kDa (in recipients of the $V_HC\alpha$ chimeric genes) and 42 kDa (in $V_HC\beta$ recipients) are also apparent, representing the non-S—S linked, monomer chimeric chains present in the cell lysates. Similar blots of gels electrophoresed under reducing conditions or developed with a radioiodinated anti-mouse Fab' antibody failed to give any signal. The heterogeneity in size of the chimeric α/β heterodimer that was also observed in immunoprecipitation analysis of surface labeled receptors, (ref 24 and unpublished data) is most likely due to the formation of heterodimers that are formed by pairing between the chimeric chains and the complementary endogenous α or β chains of the MD.45 hybridoma. The intensity of the different bands reflects the amount of the chimeric receptor in the cell lysates and the degree of reactivity with the antiidiotypic antibody.

Functional Expression of the Chimeric TcR Genes. To examine whether the chimeric TcR genes code for a functional receptor, we tested the ability of the various transfectants to produce lymphokines in response to TNP-modified cells and to kill target cells bearing TNP groups on their surface. When TNP-A.20, (a TNP-modified B-cell lymphoma from BALB/c mice), was used to stimulate the various transfectomas, almost all of the double transfectants produced IL-2 (Fig. 3). No production of IL-2 was evident when unmodified A.20 cells were used as stimulators (data not

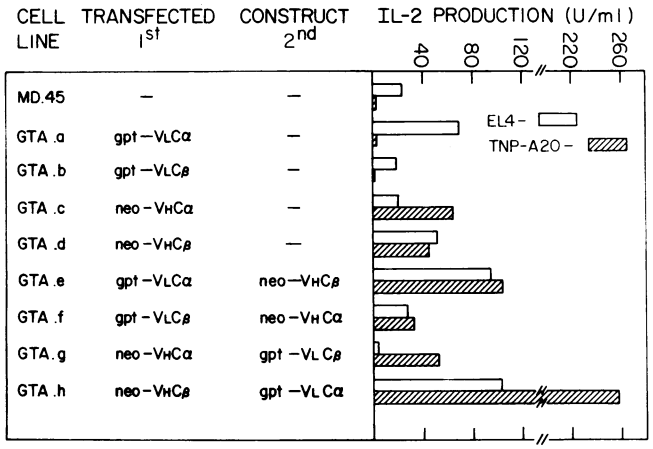

FIG. 3. Production of IL-2 by transfectants following stimulation with EL4 and TNP-modified A.20 cells.

Table 1. Transfectants expressing chimeric TcR respond to TNP-coupled cells and proteins

Cell line	Transfected chimeric gene(s)	IL-2 production following stimulation, units/ml						
		EL4	A20	TNP-A20	TNP-Spc*	TNP-ARH†	TNP-KLH	TNP-FγG
MD.45	—	17	0	0	0	0	0	0
GTA.a	$V_LC\alpha$	26	0	1	3	0	0	0
GTA.b	$V_LC\beta$	50	0	0	1	1	0	0
GTA.c	$V_HC\alpha$	30	0	71	11	1	0	0
GTA.d	$V_HC\beta$	15	0	23	5	2	6	38
GTA.g	$V_HC\alpha + V_LC\beta$	2	0	33	9	8	84	136
GTA.h	$V_HC\beta + V_LC\alpha$	72	0	108	10	20	150	320

Transfectants were stimulated with x-irradiated target cells at a 1:1 ratio or with 2 μg of TNP-coupled proteins per well adsorbed to the wells of microtiter plate. KLH, keyhole limpet hemocyanin; FγG, fowl gamma globulin.
*Spleen cells of SJL/J mice.
†Human lymphoblastoid cell line.

shown) or when MD.45 hybridoma cells or transfectants that expressed only the chimeric $V_LC\alpha$ (or $C\beta$) constructs were used as responders (GTA.a and GTA.b, Fig. 3). These cells were able to produce IL-2, as was evident by their ability to respond to EL-4 (the H-2^b target cell of the MD.45 hybridoma). Interestingly, transfectants that received and expressed the $V_HC\alpha$ (GTA.c) or $V_HC\beta$ (GTA.d) chimeric genes could be stimulated by TNP-A.20 cells, suggesting that the TNP-binding capacity, like the Sp6 idiotype, resides primarily within the Sp6 V_H domain.

The cTcR recognized TNP in a non-MHC-restricted manner, as evident by the ability of the transfectants to respond to TNP-modified allogeneic and xenogeneic cells (Table 1). Transfectants bearing the chimeric TcR could be activated by TNP groups bound directly to stimulator cells (Fig. 3, Table 1) or coupled to a protein carrier (such as TNP-bovine serum albumin) and presented either by antigen-presenting cells (APCs) such as A.20 and spleen cells (not shown) or adsorbed onto plastic substrate (Table 1). TNP-modified proteins in solution inhibited specifically and in a dose-dependent manner the activation of the various transfectants by immobilized antigen (Fig. 4*A*), indicating that, like antibodies, the cTcR was able to bind soluble antigen. Anti-SP6-idiotype as well as anti-TNP mAbs also inhibited completely the IL-2 production by the cells (Fig. 4 *B* and *C*).

Another manifestation of the functional expression of the chimeric receptor, in the effector phase of T-cell response, is demonstrated by the ability of the transfectants to specifically kill haptenated target cells as measured by the ^{51}Cr-release assay. As shown in Fig. 5, all cells studied (except GTA.g2, which lost its ability to recognize EL-4 cells) killed EL-4 as well as TNP-EL4 cells, and their lytic ability was increased following prestimulation with EL-4. However, only the transfectants could kill the TNP-A.20 target cells. Accordingly, stimulation with TNP-A.20 cells enhanced only the reactivity of cells that expressed the chimeric TcR. Similar results have been obtained when TNP-438, *H-2*^{s} B-lymphoma cells were used as targets in the killing assay (data not shown). These studies are compatible with the idea that the chimeric receptor can mediate non-MHC-restricted, antigen-specific target cell lysis.

DISCUSSION

In this article we have shown that chimeric TcR chains, composed of immunoglobulin V regions and the TcR C regions, can be functionally expressed in T cells. Expression of this cTcR endowed the recipient T cell with antibody-type specificity: it could recognize and respond to stimulator and target cells that bear the TNP haptenic group on their surfaces, as evident by either IL-2 production (Table 1, Fig. 3) or cytolytic activity (Fig. 5). The recognition of TNP is non-MHC restricted, as manifested by the ability of recipients of the chimeric genes to respond to TNP-modified cells of different strains and species and to TNP adsorbed onto plastic, and is independent of the endogenous TcR of the recipient hybridoma. The antibody-derived origin of the cTcR is further supported by the observation that the anti-Sp6 mAb 20.5 reacted with the chimeric receptor in immunoblotting (Fig. 2*B*) and immunoprecipitation (24) and completely inhibited the activity of most of the transfectants toward TNP (Fig. 4). Finally, like their parental antibody, Sp6, the cTcRs also bind TNP-modified antigens in solution (Fig. 4*A*). The ability of soluble antigens to inhibit the stimulation of the transfectants provides us with direct methods to study the effect of physicochemical parameters, such as receptor affinity, and antigenic valency, density, and rigidity on T-cell activation.

The Sp6 antibody-binding site is expressed on the transfectants regardless of which immunoglobulin V region was combined with which TcR C region domains. Both $V_HC\alpha$ and $V_LC\beta$ or $V_HC\beta$ and $V_LC\alpha$ chain combinations could pair,

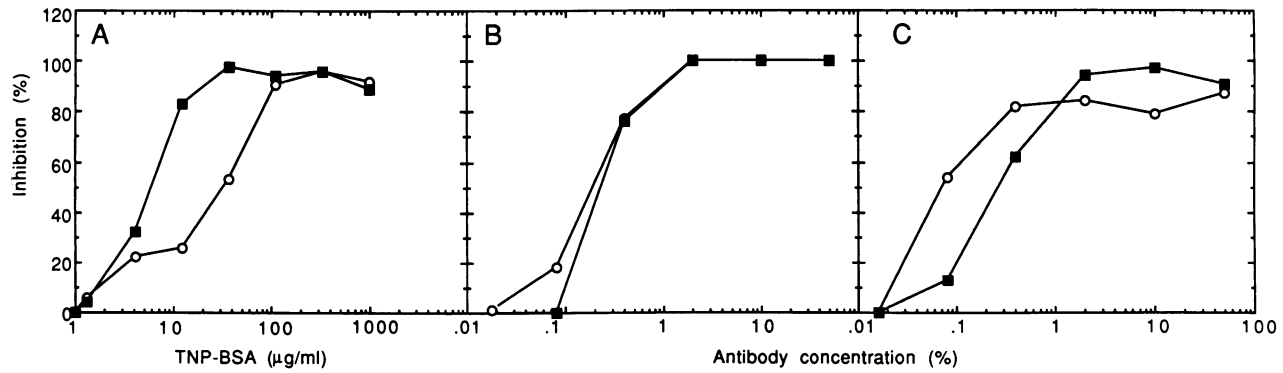

FIG. 4. Inhibition of IL-2 produced by GTA.g (○) and GTA.h (■) transfectants following stimulation with TNP-A.20 cells. Different concentrations of soluble TNP_5-BSA (BSA, bovine serum albumin) (*A*), anti-Sp6 idiotypic mAb (20.5) (*B*), and anti-TNP mAb (Sp6) (*C*) were added to the reaction wells before adding the responding "transfectomas." (*B* and *C*) Culture supernatant containing the mAb.

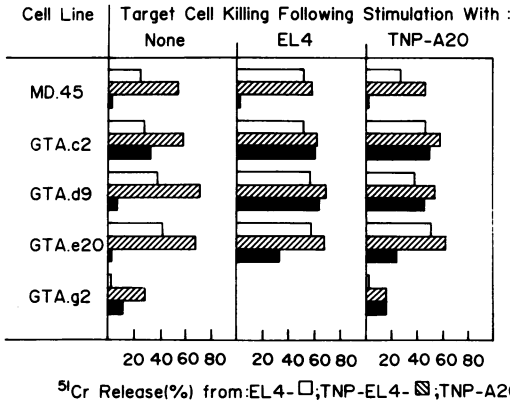

FIG. 5. Cytotoxic activity of transfectants. The different transfectants and MD.45 parental cells were incubated at an effector:target ratio of 2:1 with ^{51}Cr-labeled target cells. Effector cells were used either without stimulation or following preincubation with irradiated EL4 or TNP-A.20 stimulator cells.

form sulfhydryl bonds, assemble with T3 molecules (ref. 25 and data not shown), and be expressed in the membrane of T cells as functional dimers that bind the hapten, display the Sp6 idiotope, and transmit a signal for T-cell activation. In most of the transfectants that received both chimeric genes, a tetrameric complex of about 160 kDa was also observed (Fig. 2*B*); however, its functional significance has yet to be established. Interestingly, in the experimental system we employed, using the Sp6 V domains, it appears that the V_H by itself can account for most of the antibody-binding capacity and expression of the idiotypic determinant. Hence, transfectants receiving either $V_HC\alpha$ or $V_HC\beta$ chimeric genes alone expressed functional receptors that bind TNP (GTA.c, GTA.d, Fig. 3, Table 1) and react with the anti-Sp6 idiotypic antibody 20.5 (Figs. 2*B* and 4). It seems likely that the V_H-containing cTcR chains can pair with the complementary chain of the endogeneous TcR to yield a functional heterodimeric receptor. This was directly shown by the ability of anti-Vβ mAb to immunoprecipitate cTcRs that express both V_H and the endogenous β chain (24). The fact that the H-chain V region contributes most of the contact residues in the binding site of several antibodies was established by studies that assigned antibody activity to isolated chains (26). The structural similarity between the TcR Vα and Vβ to the immunoglobulin V_L, as manifested by the strong resemblance in their invariant amino acids (2) and distance between the cysteines that form the intrachain disulfide bond (23), may explain the efficiency by which the V_H chimeric chain pairs with either one of the endogenous TcRα or β chains to yield an active binding site.

It is noteworthy that both quantitative and qualitative differences have been observed in the degree of reactivity and the ability of the different transfectants that received various combinations of chimeric genes to respond to different forms of antigen and stimulator cells. For example, the GTA.g series that was first transfected with $V_HC\alpha$ and then with $V_LC\beta$ responded better to TNP-bovine serum albumin than did the GTA.f series that received the same chimeric gene combination in reverse order. The GTA.d series that was transfected only with $V_HC\beta$ could be better inhibited by the 20.5 mAb than the GTA.c series that received the $V_HC\alpha$ gene. We believe that these differences are due to the level of expression of the chimeric TcR and the degree of pairing with the endogenous TcR chains. It is likely that pairing of the original V_H and V_L will result in the binding site with the highest affinity and specificity toward the hapten.

Our successful attempts to express functional chimeric TcRs that recognize antigen in a non-MHC-restricted manner pave the way for an approach for the design at will of TcRs of any desired specificity, provided that such specificity can be predefined by a mAb. Our ability to combine antibody specificity with T-cell-mediated target cell lysis may have clinical potential: it enables the construction of chimeric TcR genes using the V regions of antibodies directed at desired antigens on a given target cell. These chimeric genes, once produced, are non-MHC-restricted and universal in the sense that a given set of chimeric genes could then be transfected into T cells from any individual. Upon returning the cells to their donors, they should manifest the specificity of the cTcR by proliferating and mediating specific effector function (cytolysis, production of lymphokines, help, or suppression) when encountering their target cells. This approach can be exploited, for example, to direct cytotoxic T lymphocytes to kill tumor or virally infected cells. Construction of cTcRs with anti-tumor specificity will enable testing of the feasibility of this approach in combating human tumors.

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