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Cloning and Sequencing of the V_H and V_κ Genes of an Anti-CD3 Monoclonal Antibody, and Construction of a Mouse/Human Chimeric Antibody[1]

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Mouse monoclonal antibodies against CD3 on human T lymphocytes have been used for therapy in organ-transplant patients as a potent immunosuppressive agent or for treatment of cancer as a potent T cell activating agent. However, an inherent problem in their *in vivo* application is the human anti-mouse antibody response. In this study, we cloned and sequenced the variable region genes of the heavy and light chains (V_H and V_κ) of a mouse anti-human CD3 monoclonal antibody (OKT3) using the reverse transcription-polymerase chain reaction method. Then, we constructed a mouse/human chimeric antibody, designated as Ch OKT3, by fusing the OKT3 V_H and V_κ genes to the human heavy and light chain constant region genes ($C_\gamma 1$ and C_κ) derived from a human plasma cell leukemia line (ARH77), respectively. The chimeric gene constructs were sequentially co-transfected into mouse non-Ig-producing hybridoma cells (Sp2/0) by electroporation. The Ch OKT3 antibody thus prepared bound to human peripheral blood mononuclear cells and competitively inhibited the binding of the parental MAb OKT3 to the blood mononuclear cells, indicating that this chimeric antibody seems to be suitable for *in vivo* therapeutic approaches.

Key words: CD3, chimeric antibody, monoclonal antibody, OKT3.

Hybridoma technology has yielded monoclonal antibodies (MAbs) with specificities to a wide range of antigens, including lymphocyte cell surface markers, viral coat proteins, and tumor-associated antigens. Such MAbs have great potential as agents in the diagnosis and therapy of human diseases. The human CD3 on T cells consists of a minimum of five polypeptide chains (γ, δ, ε, ζ, and η) non-covalently associated with the T cell receptor (TCR), and plays an important role in the process of T cell activation in response to antigen recognition by the TCR (*1*). So far, several mouse or rat anti-human CD3 MAbs, such as OKT3 (*2*), UCTH1 (*3*), YTH12.5 (*4*), and CLB-T3/4.1 (*5*), have been prepared for the detection and characterization of human CD3 molecules. Among these MAbs, OKT3 is a mouse MAb directed against a conformational epitope formed between CD3-ε and either CD3-γ or CD3-δ on human T lymphocytes (*6*). This MAb exhibits a potent T cell activation or suppression property *in vivo* as well as *in vitro* (*7, 8*), and is the most widely-used mouse anti-human CD3 MAb (*9*). There are several inherent problems, however, in the *in vivo* application of rodent MAbs, particularly the human immune response against xenogeneic antibodies, which sometimes prevents re-treatment with MAbs (*10*). In this context, several mouse/human chimeric antibodies or humanized antibodies to human CD3, derived from the above-mentioned MAbs, have been generated for therapy in organ-transplant or cancer patients (*11, 12*). However, the complete nucleotide sequences of the heavy chain variable (V) region genes of OKT3 have not been reported.

We recently cloned the genomic V region genes (including the promoters) of the heavy and light chains (V_H and V_κ) of two mouse MAbs (F11-35 and F11-39) against carcinoembryonic antigen, and generated mouse/human chimeric antibodies which exhibited the same high specificity and affinity for carcinoembryonic antigen as those of the parental MAbs (*13, 14*). In this study, we cloned and sequenced the V_H and V_κ cDNAs of OKT3. Then, we constructed a mouse/human chimeric antibody to CD3, designated as Ch OKT3, by using the Ig-specific promoters derived from F11-39, the amplified OKT3 V_H and OKT3 V_κ genes, and the human heavy and light chain constant (C) region genes ($C_\gamma 1$ and C_κ) derived from a human plasma cell leukemia line (ARH77). The chimeric gene constructs were

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Abbreviations: HPBMCs, human peripheral blood mononuclear cells; MAb, monoclonal antibody; TCR, T cell receptor.

expressed in mouse non-Ig-producing hybridoma cells (Sp2/0), and the immunochemical properties of the resultant chimeric antibody were compared with those of the parental mouse MAb.

MATERIALS AND METHODS

Reagents—[^{35}S]Methionine was obtained from Amersham Life Science (Buckinghamshire, England). The First-Strand cDNA Synthesis Kit was from Pharmacia Biotech (Uppsala, Sweden); Mouse Ig-Primer Set and pT7Blue T-Vector from Novagen (Madison, WI); the Sequenase™ Ver 2.0 DNA sequencing kit from United States Biochemical (Cleveland, OH); mycophenolic acid and G-418 sulfate from Gibco BRL (Grand Island, NY); xanthine and tunicamycin from Sigma (St. Louis, MO); affinity-purified goat anti-human IgG(H+L) antibody from Vector (Burlingame, CA); affinity purified goat anti-mouse IgG antibody from EY Laboratories (San Mateo, CA); fluorescein-conjugated affinity purified goat anti-human IgG F(ab')$_2$ fragments and fluorescein-conjugated affinity purified sheep anti-mouse IgG antibody from Cappel (Turnhout, Belgium); human IgG1(κ) myeloma protein from The Binding Site (Birmingham, England); and Activated Immunoaffinity Support AF-Tresyl Toyopearl 650M from Tosoh (Tokyo).

Hybridoma and Myeloma Cell Lines—Hybridoma cell line OKT3 secretes a mouse IgG2a(κ) MAb directed against the human CD3 complex (*2*), and was obtained from the American Type Culture Collection (Rockville, MD). Mouse non-secreting myeloma cell line Sp2/0-Ag14 (Sp2/0) was used for transfection of the chimeric genes. All cell lines were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum.

Cloning and Sequencing of OKT3 cDNAs—Total RNA was isolated from a hybridoma cell line, OKT3, by the acid guanidinium thiocyanate-phenol-chloroform extraction method (*15*). Then, the V_H and V_κ genes of OKT3 were cloned using the reverse transcription-polymerase chain reaction (RT-PCR) method (*16*). Briefly, first-strand cDNA was prepared from the total RNA with reverse transcriptase in the First-Strand cDNA Synthesis Kit, and the cDNAs encoding the V_H and V_κ regions of OKT3 were specifically amplified by PCR using the primer sets in the Mouse Ig-Primer Set in conjunction with Taq DNA polymerase. The resulting PCR products were cloned into the pT7Blue plasmid vector. The selected plasmid DNAs were characterized by restriction endonuclease mapping, and sequenced with single- or double-stranded templates by the dideoxynucleotide chain termination method using the Sequenase™ DNA sequencing kit.

Transfection of DNA into Mouse Myeloma Cells—The chimeric heavy and light chain expression construct genes were inserted into the pSV2gpt and pSV2neo plasmid vectors, respectively (*17*). After linearization, 20 μg each of the chimeric heavy and light chain expression plasmids was co-transfected into mouse myeloma cell line Sp2/0 using an electroporation apparatus (Gene Pulser; Bio-Rad Lab., Richmond, CA, USA), as described previously (*18*). The transfected cells were incubated at 37°C under 5% CO_2 for 72 h in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, and then transferred to selective medium containing mycophenolic acid (6.5 μg/ml) and xanthine (250 μg/ml) for the selection of cells containing gpt-resistant plasmids, and G418 (1 mg/ml) for cells containing neo-resistant plasmids. Transfectoma clones were cultured at 37°C in Dulbecco's modified Eagle's medium containing 10% fetal calf serum.

Biosynthetic Labeling and Immunoprecipitation—The hybridoma and transfectomas were cultured for 6 h with 1.85 MBq/1.5 ml of [^{35}S]methionine in methionine-free modified Eagle's medium in the presence or absence of 5 μg/ml tunicamycin. The secreted monoclonal or chimeric immunoglobulins were precipitated from spent culture medium with goat anti-human IgG(H+L) or mouse IgG antibody coupled to AF-Tresyl Toyopearl, and then analyzed by SDS-PAGE followed by fluorography (*19*).

Human IgG Activity—Quantitation of chimeric antibodies was carried out by means of a solid-phase enzyme immunoassay using 96-well polyvinyl chloride plates (Greiner GmbH, Frickenhausen, Germany) coated with an affinity-purified goat anti-human IgG(H+L) antibody and a biotinylated goat anti-human γ-chain antibody as the tracer (*13*). A purified human IgG1(κ) myeloma protein was used as the standard.

Preparation of Human Peripheral Blood Mononuclear Cells—Human peripheral blood mononuclear cells (HPBMCs) were obtained from heparinized peripheral blood of healthy human donors as described previously (*20*). Isolation was performed by density gradient centrifugation of diluted (1:1 PBS) blood on Lymphoprep (Nycomed, Oslo, Norway) at 1,500 rpm for 30 min. HPBMCs collected from the gradient interphase were washed repeatedly with PBS to remove platelets.

Flow Cytometry—To determine antibody binding to HPBMCs, flow cytometry was performed (*21*). After washing with ice-cold PBS containing 5% normal goat serum (wash buffer), aliquots of 2×10^6 HPBMCs were mixed with 200 μl of chimeric or parental monoclonal OKT3 (2.5 μg each/ml in PBS containing 5% normal goat serum). The HPBMCs were incubated on ice for 1 h, washed three times, and then stained with fluorescein-conjugated goat (Fab')$_2$ anti-human IgG or sheep anti-mouse IgG on ice for 1 h. Then, the stained cells were washed, resuspended in 1.0 ml FACSFlow medium, filtered through a nylon mesh, and analyzed with a FACScan (Becton Dickinson, Mountain View, CA). In a competitive inhibition assay, HPBMCs were treated with the parental MAb OKT3 in the absence or presence of Ch OKT3 (2.5 μg/ml) before the addition of fluorescein-conjugated sheep anti-mouse IgG.

RESULTS

Cloning, Sequencing, and Amplification of V Region cDNAs of OKT3—The cDNAs encoding the V_H and V_κ regions of OKT3 were cloned with the RT-PCR using First-Strand cDNA Synthesis Kit and Mouse Ig-Primer Set. The nucleotide sequences and deduced amino acid sequences of the V_H and V_κ genes are shown in Fig. 1.

The selected V_H and V_κ genes of OKT3 were amplified and cloned by a second PCR, followed by ligation into the pT7Blue plasmid vector. The primers including appropriate terminal restriction sites, which are added to the V_H and V_κ sequences in Fig. 1, were prepared in this study and used for the second PCR. The amplified OKT3 V_H and OKT3 V_κ genes were digested with *Nco*I/*Nsi*I and *Nco*I/*Afl*II, re-

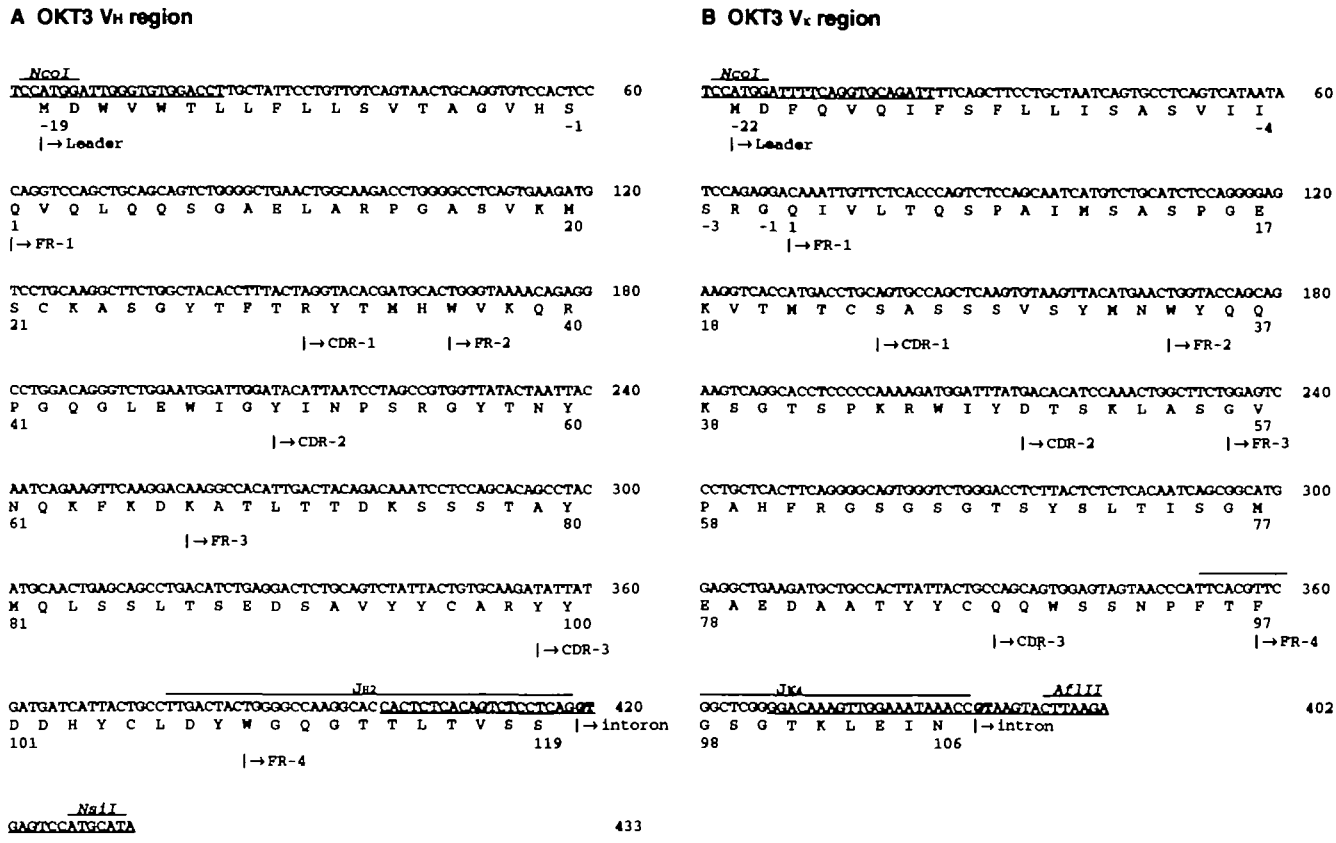

Fig. 1. **Nucleotide sequences and deduced amino acid sequences of the V_H region (A) and V_κ region (B) genes of OKT3.** The primer sequences including appropriate terminal restriction sites for second PCR added to the nucleotide sequences are underlined. The restriction sites are shown in underlined italic type over the nucleotide sequences. FR, framework; CDR, complementarity-determining region. The rearranged J_H2 (A) and $J_\kappa4$ (B) regions are given over the nucleotide sequences. The letters in bold face show the donor splicing sites of intron.

spectively, and then purified by agarose gel electrophoresis.

Construction of Chimeric Antibody Expression Vectors—The Ig-specific promoters of the mouse Ig genomic V_H and V_κ genes, which had been prepared from a F11-39 hybridoma (*13, 14*), and ligated into the pUC118 and pUC119 plasmid vectors, respectively, were used in this study. The *Hin*dIII site of the 1.2 kb F11-39 mouse Ig V_H (*Eco*RI/*Hin*dIII) fragment was changed to a *Eco*RI site (Fig. 2A), and the 1.3 kb *Sph*I-*Sty*I fragment of the F11-39 V_κ gene was used after site conversions from *Sph*I to *Eco*RI and from *Sty*I to *Bam*HI (Fig. 2B). A *Nco*I site was introduced into the F11-39 V_H and V_κ genes by site-mutagenesis at each initiation codon, respectively. Then, the F11-39 V_H and F11-39 V_κ genes were replaced by the amplified OKT3 V_H and OKT3 V_κ genes, respectively (Fig. 2, A and B).

The OKT3 V_H and OKT3 V_κ genes containing the mouse Ig-specific promotor genes were ligated to the human $C_\gamma1$ and C_κ genes from a human plasmacytoma line, ARH77 (*22*), in the pSV2gpt and pSV2neo plasmid vectors (*17*), respectively (Fig. 2, A and B). These expression vectors also contained human heavy chain enhancer elements.

Production of Chimeric Antibody—The chimeric heavy and light chain expression plasmids were linearized with *Bam*HI and *Aat*II in a nonessential part of the vector, respectively. These chimeric heavy and light genes were co-transfected into the mouse hybridoma cells, Sp2/0, by electroporation. After selection with mycophenolic acid, xanthine and G418, the cell supernatants were screened for human IgG production by means of the solid-phase enzyme immunoassay, and the transfectomas were cloned by the limiting dilution technique as described previously. The highest producer was cultured for antibody production and the resultant chimeric antibody was designated as Ch OKT3. The amount of Ch OKT3 in the culture supernatant was about 1,500 ng/10^6 cells/ml/24 h.

SDS-PAGE Analysis of Ch OKT3—The translation products of the Sp2/0 transfectoma and mouse OKT3 hybridoma were labeled with [^{35}S]methionine, immunoprecipitated with the anti-human IgG(H+L)- or anti-mouse IgG-Toyopearl, and then analyzed by SDS-PAGE. Under nonreducing conditions, the precipitates gave a band at a portion corresponding to the assembled protein of H_2L_2, respectively, but the molecular weight (160 kDa) of Ch OKT3 was a little higher than that (150 kDa) of the parental OKT3 (Fig. 3A). Under reducing conditions, two bands appeared at positions corresponding to the heavy and light chains (Fig. 3B, lanes 1 and 2). The molecular weight (52 kDa) of the chimeric heavy chain was similar to that of the parental mouse one. In contrast, the molecular weight (27 kDa) of the Ch OKT3 light chain, which appeared to give a double band, was higher than that of the parental OKT3 (22 kDa). When the Sp2/0 transfectoma and mouse OKT3 hybridoma were treated with the *N*-glycosylation inhibitor, tunicamycin (TM), the mobilities of the Ch

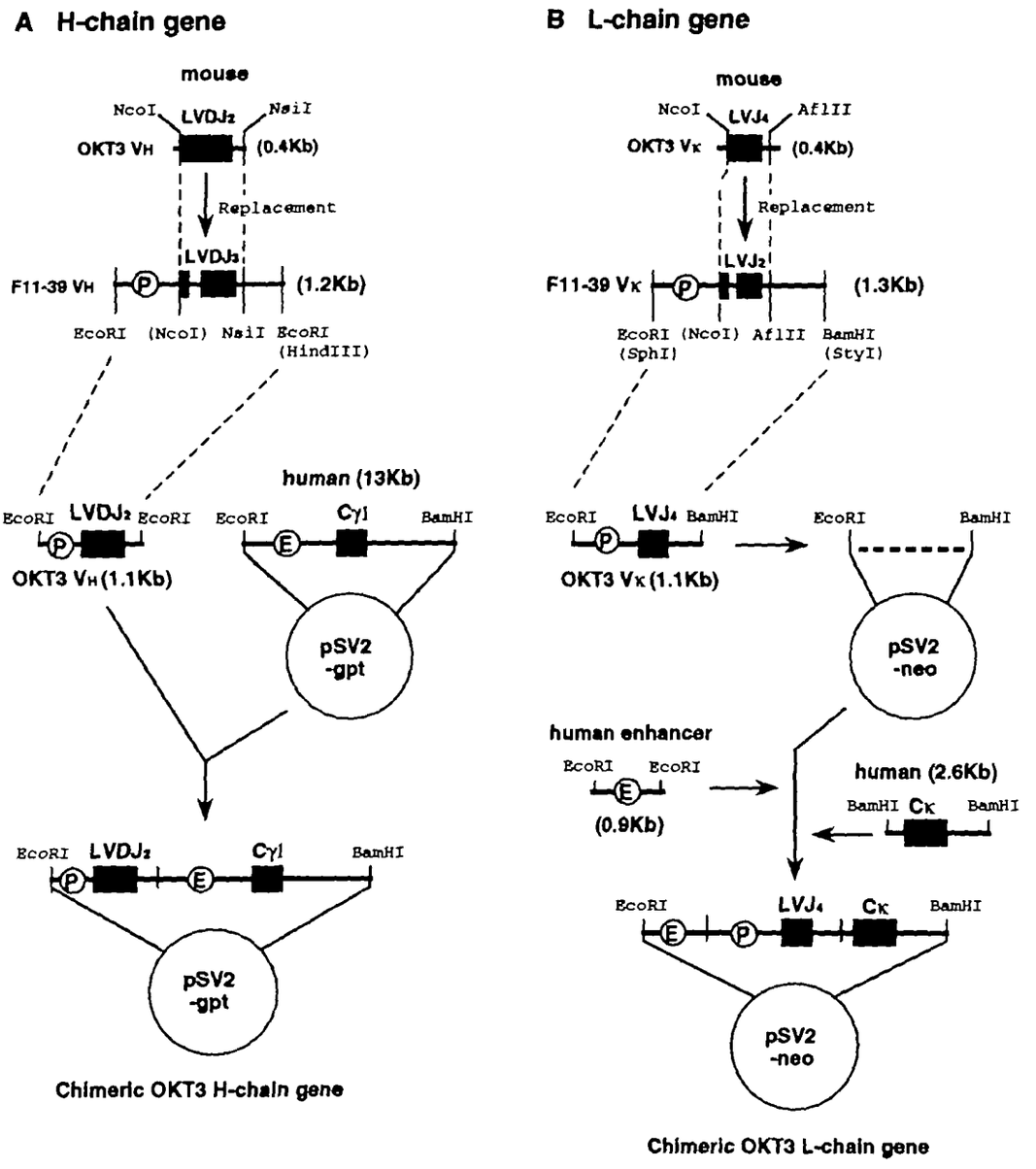

Fig. 2. **Construction of chimeric OKT3 H-chain (A) and OKT3 L-chain (B) expression vectors.** L, leader segment; V, variable region; D, diversity segment; J, joining segment; P, mouse promotor; E, human enhancer. Exon regions of the human and mouse Ig heavy and light chain genes are shown by solid boxes.

OKT3 and parental OKT3 heavy chains slightly increased (Fig. 3B, lanes 3 and 4). The mobility of the parental OKT3 light chain remained almost unchanged (Fig. 3B, lane 3), whereas that of the chimeric light chain, which turned into a single band, remarkably increased, its molecular weight being about 23 kDa (Fig. 3B, lane 4).

Binding Reactivity of Ch OKT3 to Human Lymphocytes—The binding of Ch OKT3 to HPBMCs was investigated by flow cytometric analysis (Fig. 4). Ch OKT3 significantly bound to HPBMCs, as shown by the significant increase in the fluorescence intensity compared with the background level obtained with medium only, and the fluorescence intensity of Ch OKT3 was comparable to that of the parental MAb OKT3 (Fig. 4A). The presence of Ch OKT3 in the binding assay reduced the binding of OKT3 to almost the background level (Fig. 4B).

DISCUSSION

MAbs against CD3 can activate T cells in the absence of antigen. An additional signal provided by accessory cells (*i.e.* monocytes), however, is required to trigger T cell proliferation. Monocytes cross-link anti-CD3 MAbs *via* their Fc receptors (*23*), and secrete lymphokines such as IL-1 or IL-4 (*8, 24*). On the other hand, in the absence of cross-linking, capping of TCR/CD3 occurs on challenge with anti-CD3, which results in a state of T-cell unresponsiveness (*25*). Thus, MAbs against CD3 can be used to

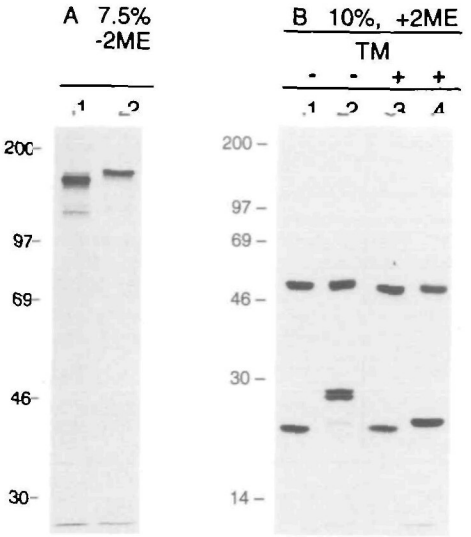

Fig. 3. **SDS-PAGE analysis of translated products of the Sp2/0 transfectoma and murine OKT3 hybridoma.** The transfectoma and OKT3 hybridoma were cultured for 6 h with 1.85 MBq/1.5 ml of [^{35}S]*methionine*. The secreted Ig in the culture supernatants was precipitated with goat anti-human IgG(H+L)- or anti-mouse IgG-AF-Tresyl Toyopearl, and then electrophoresed on a 7.5% gel without reduction (A), or a 10% gel with reduction by 2-mercaptoethanol (2ME) (B), followed by fluorography. TM, tunicamycin-treated. A: lane 1, OKT3; and lane 2, Ch OKT3. B: lanes 1 and 3, OKT3; lanes 2 and 4, Ch OKT3. Vertical markers, molecular weight markers ($\times 10^{-3}$).

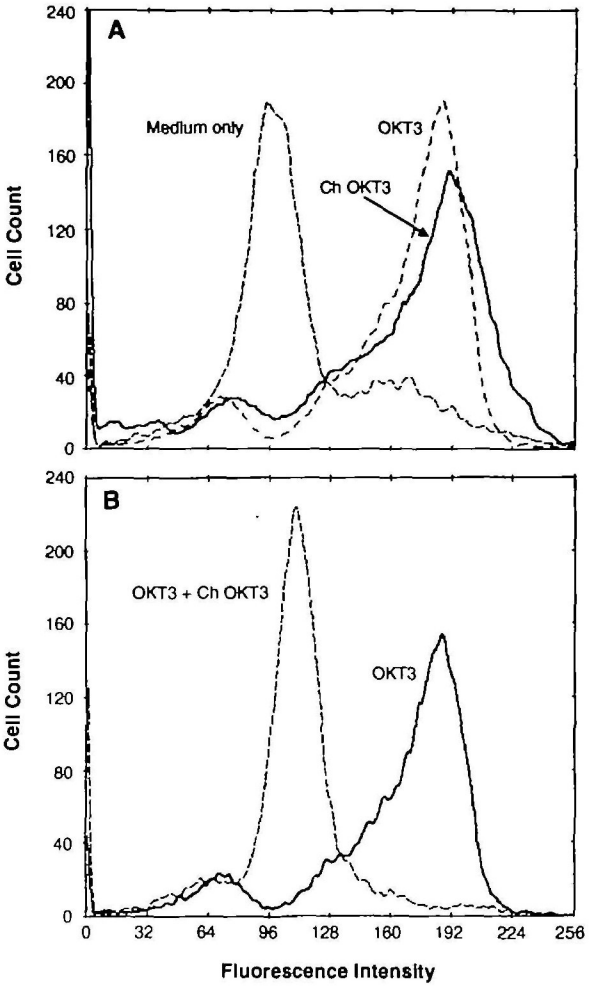

Fig. 4. **Flow cytometric analysis of the binding of Ch OKT3 to HPBMCs.** (A) HPBMCs were incubated with the medium only, Ch OKT3, or OKT3. Then, the HPBMCs were stained with fluorescein-conjugated goat F(ab')$_2$ anti-human IgG or fluorescein-conjugated sheep anti-mouse IgG. (B) HPBMCs were incubated with OKT3 in the absence or presence of Ch OKT3. Then, the HPBMCs were stained with fluorescein-conjugated sheep anti-mouse IgG.

influence the immunological status by enhancing or suppressing T cell responses to antigens. Furthermore, bispecific MAbs with dual specificities for a tumor-associated antigen and for CD3 on immune effector cells have also been developed as new agents for immunotherapy, and have proved effective for retargeting effector cells to kill tumor cells both *in vitro* and *in vivo* (*26, 27*). Therefore, MAbs against CD3 on human T cells have considerable therapeutic potential and have been used extensively in humans as immunosuppressive or immunopotentiating agents (*10, 28*). However, most of them are of murine origin, and thus there are the problems of strong immunogenicity and rapid serum clearance in humans (*29, 30*). In this study, we cloned and sequenced the V_H and V_κ genes of a mouse anti-human CD3 MAb (OKT3), and constructed a mouse/human chimeric antibody, designated as Ch OKT3, by fusing the OKT3 V_H and V_κ genes to the human $C_\gamma 1$ and C_κ genes, respectively. The complete nucleotide sequences of the V_H and V_κ region genes of OKT3 are first determined in this study (Fig. 1), demonstrating the V_H and V_κ genes of OKT3 had been rearranged to the $J_H 2$ and $J_\kappa 4$ exons, respectively (*31*).

When determined by SDS-PAGE, the molecular weight of the resultant chimeric antibody, Ch OKT3, was a little higher than that of the parental MAb OKT3. The difference in molecular weight was due to the different degrees of glycosylation of light chains because the molecular weight of the Ch OKT3 light chain was remarkably higher than that of the OKT3 light chain, and it decreased upon treatment of transfected cells with tunicamycin. This suggests that a possible *N*-glycosylation site (Asn/Arg/Thr) newly appeared at the combining position between the mouse V_κ region and the human C_κ region of Ch OKT3. In fact, the C-terminal amino acid of the OKT3 V_κ region was Asn (N), as shown in Fig. 1, and the first two-residue amino acid sequence at the N-terminus of the human C_κ region was Arg and Thr (*31*). On the other hand, the first two-residue amino acid sequence at the N-terminus of the mouse C_κ region, which is the C region of the OKT3 light chain, was Arg and Ala (*31*), implying no *N*-glycosylation site at the combining position between the V_κ and C_κ regions of OKT3. In spite of the glycosylation at the light chain V_κ and C_κ combining position, Ch OKT3 bound to human peripheral blood mononuclear cells and competitively inhibited the binding of the parental MAb OKT3 to the blood mononuclear cells.

The above results taken together indicate the chimeric antibody, Ch OKT3, may be a potential useful therapeutic reagent for the treatment of rejection episodes following organ transplantation or for the treatment of cancer by cross-linking with a chimeric antibody against tumor-associated antigens.

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REFERENCES

1. Roitt, I.M. (1994) Membrane receptors for antigen in *Essential Immunology*, 8th ed., pp. 64-80, Blackwell Scientific Publications, London
2. Kung, P.C., Goldstein, G., Reinherz, E.L., and Schlossman, S.F. (1979) Monoclonal antibodies defining distinctive human T cell surface antigens. *Science* **206**, 347-349
3. Beverley, P.C.L. and Callard, R.E. (1981) Distinctive functional characteristics of human "T" lymphocytes defined by E rosetting or a monoclonal anti-T cell antibody. *Eur. J. Immunol.* **11**, 329-334
4. Clark, M., Bindon, C., Dyer, M., Friend, P., Hale, G., Cobbold, S., Calne, R., and Waldmann, H. (1989) The improved lytic function and in vivo efficacy of monovalent monoclonal CD3 antibodies. *Eur. J. Immunol.* **19**, 381-388
5. Tunnacliffe, A., Olsson, C., Traunecker, A., Krissansen, G.W., Karjalainen, K., and de la Hera, A. (1989) The majority of CD3 epitopes are conferred by the ϵ chain in *Leukocyte Typing IV; White Cell Differentiation Antigens* (Knapp, W., Gilks, W.R., Rieber, E.P., Schmidt, R.E., Stein, H., and Von dem Borne, A.E.G. Kr., eds.) pp. 295-296, Oxford University Press, Oxford
6. Salmeron, A., Sanchez-Madrid, F., Ursa M.A., Fresno, M., and Alarcon, B. (1991) A conformational epitope expressed upon association of CD3-epsilon with either CD3-delta or CD3-gamma is the main target for recognition by anti-CD3 monoclonal antibodies. *J. Immunol.* **147**, 3047-3052
7. Landegren, U., Ramstedt, U., Axberg, I., Ullberg, M., Jondal, M., and Wigzell, H. (1982) Selective inhibition of human T cell cytotoxicity at levels of target recognition or initiation of lysis by monoclonal OKT3 and Leu-2a antibodies. *J. Exp. Med.* **155**, 1579-1584
8. Flamand, V., Abramowicz, D., Goldman, M., Biernaux, C., Huez, G., Urbain, J., Moser, M., and Leo, O. (1990) Anti-CD3 antibodies induce T cells from unprimed animals to secrete IL-4 both in vitro and in vivo. *J. Immunol.* **144**, 2875-2882
9. Kreis, H., Chkoff, N., Chatenoud, L., Debure, A., Acombe, M., Chrétien, Y., Legendre, C., Caillat, S., and Bach, J.F. (1989) A randomized trial comparing the efficacy of OKT3 used to prevent or to treat rejection. *Transplant Proc.* **21**, 1741-1744
10. Jaffers, G.J., Fuller, T.C., Cosimi, A.B., Russell, P.S., Winn, H.J., and Colvin, R.B. (1986) Monoclonal antibody therapy. Anti-idiotypic and non-anti-idiotypic antibodies to OKT3 arising despite intense immunosuppression. *Transplantation* **41**, 572-578
11. Routledge, E.G., Lloyd, I., Gorman, S.D., Clark, M., and Waldmann, H. (1991) A humanized monovalent CD3 antibody which can activate homologous complement. *Eur. J. Immunol.* **21**, 2717-2725
12. Woodle, E.S., Thistlethwaite, J.R., Jolliffe, L.K., Zivin, R.A., Collins, A., Adair, J.R., Bodmer, M., Athwal, D., Alegre, M.-L., and Bluestone, J.A. (1992) Humanized OKT3 antibodies: Successful transfer of immune modulating properties and idiotype expression. *J. Immunol.* **148**, 2756-2763
13. Arakawa, F., Haruno, M., Kuroki, M., Kanda, H., Watanabe, T., Misumi, Y., and Matsuoka, Y. (1993) Construction and expression of two mouse-human chimeric antibodies with high specificity and affinity for carcinoembryonic antigen. *Hybridoma* **12**, 365-379
14. Kuroki, M., Arakawa, F., Haruno, M., Murakami, M., Wakisaka, M., Higuchi, H., Oikawa, S., Nakazato, H., and Matsuoka, Y. (1992) Biochemical characterization of 25 distinct carcinoembryonic antigen (CEA) epitopes recognized by 57 monoclonal antibodies and categorized into seven groups in terms of domain structure of the CEA molecule. *Hybridoma* **11**, 391-407
15. Chirgwin, J.M., Prxybyla, A.E., MacDonald, R.J., and Rutter, W.J. (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**, 5294-5299
16. Couto, J.R., Blank, E.W., Peterson, J.A., and Ceriani, R.L. (1993) Cloning of cDNAs encoding the variable domains of antibody BrE-3 and construction of a chimeric antibody. *Hybridoma* **12**, 15-23
17. Nishimura, Y., Yokoyama, M., Araki, K., Ueda, R., Kudo, A., and Watanabe, T. (1987) Recombinant human-mouse chimeric monoclonal antibody specific for common acute lymphocytic leukemia antigen. *Cancer Res.* **47**, 999-1005
18. Hama-Inaba, H., Takahashi, M., Kasai, M., Shiomi, T., Ito, A., Hanaoka, F., and Sato, K. (1987) Optimum conditions for electric pulse-mediated gene transfer to mammalian cells in suspension. *Cell Struct. Funct.* **12**, 173-180
19. Bonner, W.M. and Laskey, R.A. (1974) A firm detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* **46**, 83-88
20. Kroesen, B.J., Bakker, A., van Lier, R.A.W., The, H.T., and de Leij, L. (1995) Bispecific antibody-mediated target cell-specific costimulation of resting T cells via CD5 and CD28. *Cancer Res.* **55**, 4409-4415
21. Primus, F.J., Finch, M.D., Masci, A.M., Schlom, J., and Kashimiri, S.V.S. (1993) Self-reactive antibody expression by human carcinoma cells engineered with monoclonal antibody genes. *Cancer Res.* **53**, 3355-3361
22. Burk, K.H., Drewinko, B., Trujillo, J.M., and Ahearn, M.J. (1978) Establishment of a human plasma cell line in vitro. *Cancer Res.* **38**, 2508-2513
23. Van de Winkel, J.G.J. and Anderson, C.L. (1991) Biology of human immunoglobulin G Fc receptors. *J. Leuk. Biol.* **49**, 511-524
24. Palacios, R. (1985) Mechanisms by which accessory cells contribute in growth of resting T lymphocytes initiated by OKT3 antibody. *Eur. J. Immunol.* **15**, 645-651
25. Davis, L.S., Wacholtz, M.C., and Lipsky, P.E. (1989) The induction of T cell unresponsiveness by rapidly modulating CD3. *J. Immunol.* **142**, 1084-1094
26. Titus, J.A., Garrido, M.A., Hecht, T.T., Winkler, D.F., Wunderlich, J.R., and Segal, D.M. (1987) Human T cells targeted with anti-T3 cross-linked to antitumor antibody prevent tumor growth in nude mice. *J. Immunol.* **138**, 4018-4022
27. Nitta, T., Yagita, H., Azuma, T., Sato, K., and Okumura, K. (1989) Bispecific $F(ab')_2$ monomer prepared with anti-CD3 and anti-tumor monoclonal antibodies is most potent in induction of cytolysis of human T cells. *Eur. J. Immunol.* **19**, 1437-1441
28. Hank, J.A., Albertini, M., Wesly, O.H., Schiller, J.H., Borchert, A., Moore, K., Bechhofer, R., Storer, B., Gan, J., Gambacorti, C., Sosman, J., and Sondel, P.M. (1995) Clinical and immunological effects of treatment with murine anti-CD3 monoclonal antibody along with interleukin 2 in patients with cancer. *Clin. Cancer Res.* **1**, 481-491
29. Schroff, R.W., Foon, K.A., Beatty, S.M., Oldham, R.K., and Morgan, A.C., Jr. (1985) Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res.* **45**, 879-885
30. Courtenay-Luck, N.S., Epenetos, A.A., Moore, R., Larche, M., Pectasides, D., Dhokia, B., and Ritter, M.A. (1986) Development of primary and secondary immune responses to mouse monoclonal antibodies used in the diagnosis and therapy of malignant neoplasms. *Cancer Res.* **46**, 6489-6493
31. Kabat, E.A., Wu, T.T., Perry, H.M., Gottesham, K.S., and Foeller, C. (1991) Kappa light constant chains in *Sequences of Proteins of Immunological Interest*, 5th ed., pp. 647-652, United States Department of Health and Human Services, Washington, DC