Production and Characterization of a Murine/Human Chimeric Anti-Idiotype Antibody That Mimics Ganglioside

Alice Hastings, Sherie L. Morrison, Shinji Kanda, Romaine E. Saxton, and Reiko F. Irie

ABSTRACT

The VH and VL from a murine anti-idiotype antibody that mimics ganglioside have been cloned, sequenced, and expressed as a chimeric mouse/human IgG1 antibody. The chimeric antibody retained a binding specificity indistinguishable from the original murine antibody. The VH was a member of Vgam 3.8 family. The sequences are discussed in terms of ways in which proteins may mimic ganglioside epitopes.

INTRODUCTION

Since our initial report that gangliosides are immunogenic tumor cell surface antigens in cancer patients (1, 2), we have attempted to use monoclonal antibodies against several tumor-associated gangliosides in immunotherapy. Human IgM monoclonal antibodies that specifically recognize different gangliosides of human malignant melanoma were developed in this laboratory (3, 4). These antibodies induced complete regression of recurrent cutaneous melanoma after intratumor injection (5, 6). Our goal now is to extend our studies into active immunotherapy using ganglioside vaccines. However, ganglioside vaccines have several significant drawbacks: (a) tumor-associated gangliosides are present only in limited quantities and are relatively difficult to purify; and (b) in general, purified gangliosides are poorly immunogenic.

One way to produce better tumor specific immunogens is to exploit the idiotypic network. In this approach, the epitope responsible for eliciting tumor specific immunity is represented by an idiotype determinant present on an antibody molecule. In the case of ganglioside antigens, the anti-idiotype antibody is produced to an anti-ganglioside antibody. The ganglioside epitope, a carbohydrate determinant, is now represented by a protein epitope. In this context, the epitope should be more immunogenic because it should elicit a T-cell dependent immune response. Additionally, the antigen is now available in potentially unlimited quantities and is easy to purify.

Recently we have produced several murine monoclonal antiidiotype antibodies specific for L612, a human monoclonal antibody that recognizes ganglioside GM3 on human melanoma (7). One, designated 4C10, bears the internal image of ganglioside L612, a human monoclonal antibody, and will elicit an anti-GM3 antibody response when injected into syngeneic mice (7). Thus, the protein epitope mimics a carbohydrate determinant and can act as a surrogate antigen to induce GM3 specific antibodies.

We now describe the cloning and characterization of the VH and VL from this murine antibody and their expression as a murine/human chimeric antibody. The VH of the anti-idiotype antibody is a member of the under-represented Vgam 3.8 family (8). The structure of the variable region of the antibody is discussed in terms of ways in which the protein may mimic the carbohydrate determinant. This chimeric antibody has potential as a human therapeutic agent if the principle response it elicits in humans is anti-idiotype and hence antiganglioside. In addition, with the cloned genes available, they can now be manipulated to improve the potential efficacy of this protein as a tumor specific vaccine.

MATERIALS AND METHODS

Polymerase Chain Reaction Cloning of the Variable Regions of 4C10

**Figure 1.** The VH and VL of the 4C10 antibody were amplified by the polymerase chain reaction (11) and inserted into Bluescript KS+ to facilitate cloning. The VH was a member of the under-represented Vgam 3.8 family. The sequences are discussed in terms of ways in which proteins may mimic ganglioside epitopes.
RESULTS

Cloning of V<sub>H</sub> and V<sub>λ</sub> cDNA Sequences. Direct sequencing of mRNA prepared from 4C10 with a murine C<sub>H</sub> primer indicated that the light chain used J<sub>1</sub>; the sequence of FR3 indicated that the light chain was in the V<sub>III</sub> group of Kabat et al. (13). Many members of that group share similar or identical leader sequences. Therefore, a consensus leader primer was synthesized (ATGGAGACAGACACACTC) and in conjunction with a J<sub>1</sub> primer was used to amplify mRNA, which had been reverse transcribed using a C<sub>H</sub> primer. Sequencing of 2 clones revealed one that was a productively rearranged light chain and used J<sub>1</sub>, except that Thr 102 (ACC) was replaced by Ser (TCC). In the data base of Kabat et al. (13), there is no other example of this conservative substitution. The light chain belongs to the V<sub>2</sub> family of Potter et al. (14); all members of this family contain 39 variable region residues up to invariant Trp 35 and thus all have a CDR1 of 15 amino acids. Search of the nucleic acid data base yielded one germ-line V<sub>λ</sub> gene with only 6 nucleotides different from the V<sub>λ</sub> of the anti-idiotype (15). Three of these changes are silent (Fig. 1); at position 21 Met replaces Ile, at position 29 Val replaces Gly, and at position 66 Glu replaces Gln. One of the substitutions is within a complementarity-determining region, while the other two are within frameworks.

Before cloning the heavy chain, we first determined the sequence of the entire V<sub>H</sub> mRNA using a mouse primer and a set of intermediate primers constructed based on partial sequence determination. Using the appropriate PCR primers the V<sub>H</sub> was amplified, cloned into both Bluescript and the expression vector, and sequenced (Fig. 2). The results show that V<sub>H</sub>
uses JH3; however, for the first residue the T normally present substitution occurred is outlined. VH of 4C10 are shown under the nucleotide sequence. Amino acid substitutions

Comparison of the VH and the beginning of JH is GGCGAAGGTCACGCGTGG. This of terminal transferase. The sequence between the end of VH and the glycine residue. The G addition is consistent with the action

extra flexibility of this region should result as a consequence of this replacement, the bulky side chain of Trp is removed, and aspartic acids is replaced by a pair of glutamic acids.

underlined amino acids, the complementarity determining region. Solid with the action of terminal transferase. Comparison of the VH

4C10. Underlined amino acids, the complementarity determining region. Solid with the action of terminal transferase. The sequence between the end of VH and the glycine residue. The G addition is consistent with the action

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transfection and expression of chimeric anti-idiotype. The

anti-Id affinity. Fig. 5 shows the binding of TVE1 and 4C10 reacted only with L612 and did not react with other 3 light chains (Fig. 4b). Western blot analysis showed that the TVE1 chimeric antibody, unlike 4C10, reacted with anti-human IgG, but not with anti-murine IgG (Fig. 4, c and d). Like the original murine 4C10 antibody, the chimeric antibody showed specific anti-idiotype reactivity with the human L612 monoclonal (Fig. 4e).

the specificity of the TVE1 chimeric antibody was also confirmed by ELISA. Fig. 5 shows the binding of TVE1 and 4C10 to L612 (anti-GM3), to L72 (a human monoclonal antibody to ganglioside GD2) (3), and to human polyclonal IgM. TVE1 and 4C10 reacted only with L612 and did not react with L72 or human polyclonal IgM. The results were confirmed using a reversed ELISA in which the plates were coated with L612 and the binding of TVE1 and 4C10 tested alone or by competition (Fig. 6). Both TVE1 and 4C10 exhibited the expected concentration-dependent binding. In competitive binding assays, TVE1 and 4C10 displayed reciprocal inhibition with L612 at almost identical concentrations, suggesting equivalent anti-Id affinity.

the specificity of chimeric TVE1 was further examined by a cell-ELISA inhibition assay using melanoma cell line UCLASO-M12, which expresses ganglioside GM3 on the cell membrane at high density (Fig. 7). ELISA plates were coated with melanoma cells and inhibition of binding of L612 by TVE1 and 4C10 antibodies tested. Both chimeric TVE1 and 4C10 antibodies inhibited the binding of L612 to the tumor cell membranes to a similar extent (Fig. 7). Taken together, these results show that the anti-Id specificity of the chimeric mouse/human antibody TVE1 is indistinguishable from that of the original mouse 4C10 monoclonal antibody and that the chimeric Ab should therefore bear the internal image of ganglioside GM3.

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Comparison of the 10 sequences from that gene family with the most similar sequence with the heavy chain of 4C10 reveals 6 positions at which amino acid substitutions have taken place compared to the prototype sequence (Fig. 2). Two substitutions occur in FR3, in which Ser 76 is replaced by Asn, and Gln 81 is replaced by Leu. The other 4 substitutions occur within CD2, in which Tyr 53 is replaced by Asn and Ala 60 by Thr (hydrophobic to polar), and at positions 61 and 62, where a pair of aspartic acids is replaced by a pair of glutamic acids.

transfection and expression of chimeric anti-idiotype. the PCR generated VH and VJ clones into drug marked expression vectors were simultaneously transfected into nonproducing myeloma cell lines by electroporation and stably transfected cells selected. Transfectomas producing both chimeric heavy and light chains were identified, and one clone, TVE1, was amplified for further analysis. To characterize the chimeric protein, the transfectedoma was labeled by growth in 35S-methionine, cytoplasmic extracts and secretion prepared, and the Ig precipitated using rabbit anti-human Fab and S. aureus protein A. The precipitates were analyzed by SDS-polyacrylamide gel electrophoresis both before and after reduction of the disulfide bonds (data not shown). The chimeric heavy and light chains were of the expected molecular weights but with the light chain showing slightly reduced mobility compared to other chimeric light chains. The chimeric protein was secreted as a fully assembled H2L2 molecule.

purification and characterization of chimeric anti-idiotype. analysis of the purified TVE1 protein showed no significant difference in the size of the intact IgG molecules compared to the original mouse anti-idiotype 4C10, polyclonal murine IgG, or polyclonal human IgG (Fig. 4a). However, after reduction, the light chains of TVE1 migrated slightly more slowly than the other 3 light chains (Fig. 4b). Western blot analysis showed that the TVE1 chimeric antibody, unlike 4C10, reacted with anti-human IgG, but not with anti-murine IgG (Fig. 4, c and d). Like the original murine 4C10 antibody, the chimeric antibody showed specific anti-idiotype reactivity with the human L612 monoclonal (Fig. 4e).

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4C10

G G C G A A G G T C A C G C G T G G

DSP2.4

C C T A G T A C G A C

DSP2.8

C C T A G T A C G A C

Fig. 3. Comparison of the D region of 4C10 with other D regions. Residues in the D segments also found in 4C10 are shown by bold italics. Residues in 4C10 hypothesized to result from terminal transferase addition are underlined.
DISCUSSION

Using PCR based expression vectors recently developed in the laboratory, we have now been successful in cloning and expressing a chimeric anti-idiotypic antibody that bears the internal image of GM3, a melanoma-associated ganglioside antigen. As has previously been observed for the numerous examples of chimeric antibodies produced to date, the combining specificity of the chimeric antibody is identical to that of the original murine hybridoma antibody (reviewed in Refs. 17 and 18). Both antibodies recognize the same epitope and on a weight basis compete in a reciprocal fashion (Figs. 5 and 6). The conservative Ser to Thr substitution at amino acid 27d therefore had no effect on the binding specificity. The chimeric antibody is assembled and secreted as a complete H\(_2\)L\(_2\) molecule. Its only unusual property is the apparent molecular weight of its \(\alpha\)-light chain on SDS-polyacrylamide gel electrophoresis. Light chains of the same apparent molecular weight frequently show different migration rates (19). The difference in migration rate cannot be due to N-linked carbohydrate because there are no carbohydrate addition sequences (Asn-X-Ser/Thr) within the light chain.

Unlike the original murine hybridoma, the chimeric protein contains mostly human sequences as confirmed by Western blot analysis (Fig. 4). When injected into humans, the principle immune response should be directed against the variable region. It therefore has clinical potential as an idiotypic vaccine in cancer patients, and should induce an anti-GM3 specific immune response that may be effective against tumors bearing this antigen.

A question of fundamental interest is, how is this anti-idiotypic antibody able to mimic ganglioside? Previous examples of idiotypic mimicry are more easily understood because protein structures mimic protein structures. A monoclonal anti-idiotypic antibody directed against an antibody specific for the virus neutralizing epitope on the mammalian reovirus type 3 hemagglutinin has been shown to express an internal image of the receptor binding epitope of the reovirus hemagglutinin with molecular mimicry having a structural basis in shared primary sequences. In a second case in which 2 monoclonal antibodies were raised that bore an internal image of the \(\alpha\)-allotype of rabbits (21), mimicry appeared to be achieved by amino acids in the reverse order, and it was suggested that it is the conformation of the amino acid side chains that is important. Again, it is an example of a protein mimicking a protein, so it is easy to visualize in structural terms. In our example, we must, however, explain how a protein structure can mimic a carbohydrate structure. One of the difficulties in determining the important residues is that no cognate structures have been solved.

![Fig. 5. Comparison of the reactivity of the murine and chimeric anti-idiotypic antibodies with L612. ELISA plates were coated with 80 ng/ml TVE1 (a) or 4C10 (b) in 0.1 M carbonate buffer, pH 9.6. After blocking the plates with 5% bovine serum albumin in TPBS, binding by the anti-GM3 antibody L612, the anti-GD2 monoclonal IgM L72, and polyclonal human IgM were determined over a range of concentrations.](image)
The variable region of the antibody 4C10 remains a very intriguing example of a protein mimicking a nonprotein epitope. It is possible to theorize how this mimicry may be achieved, however, until we have solved structures to compare, such discussions of mimicry must by necessity be limited to speculation. Determining the structural basis for this mimicry is an important undertaking because, unlike proteins, ganglioside antigens cannot be made using genetic engineering techniques. Therefore, there is no obvious way at this time to produce these important substances in large quantities. Peptide or protein mimics of gangliosides would be an important advance.

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