Rapid Communication J. Biochem. 128, 345–347 (2000)

Molecular Mimicry of Human Tumor Antigen by Heavy Chain CDR3 Sequence of the Anti-Idiotypic Antibody

Dong Luo,*,[1] Weimin Qi,* Jie Ma,* Yan Jun Wang,† and David Wishart†

AltaRex Corp., 1131 Dentistry-Pharmacy; and †Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, T6G 2N8 Canada

Received June 5, 2000; accepted July 5, 2000

We isolated and characterized an anti-idiotype monoclonal antibody (AR42.1) which is capable of mimicking a distinct and specific epitope of MUC-1 antigen. The cDNA sequences coding for the AR42.1 variable regions were determined. We found significant amino acid homology between complementary determining regions 3 (CDR3) in the heavy chain of AR42.1 and the determinant epitope sequence of MUC-1. This 10 amino acid sequence may represent an "internal image" of the anti-idiotype antibody to the MUC-1 antigen, and could be used for development of a MUC-1 surrogate for immunotherapy.

Key words: anti-idiotype antibody, molecular mimicry, MUC-1 antigen.

One of the major challenges in cancer therapy is that cancer patients are developing tolerance to tumor-associated antigens during antigen-based immunotherapy. The use of an anti-idiotype antibody vaccine that acts as a surrogate for the tumor antigen to activate specific immunity in cancer patients has been reported (*1*, for review). This idea is based on the idiotypic network hypothesis (*2*), in which the infused antibody (Ab1) may elicit an anti-idiotypic response *in vivo*. Anti-idiotypic antibodies (Ab2) and corresponding T cells (T2) against the idiotype of Ab1 may occur. Parts of variable regions of anti-idiotypic antibodies might resemble the epitope that Ab1 recognizes, *i.e.* the antigen. The structural basis for the molecular mimicry may be primary sequences that are shared or expressed at the conformational level involving either Vl or Vh domains or composite Vl and Vh determinants (*3*, *4*). The specific Ab2 molecules that bear the internal image of the antigen could be very beneficial for anti-idiotypic induction in tumor vaccine design.

Our goal is to identify a specific peptide sequence by which a small molecular based cancer vaccine can be developed. This approach is used to identify and isolate an "internal antigen image" sequence from Ab2 as a surrogate antigen. Mucin MUC-1 was chosen as a model antigen because it is the best characterized human tumor cell-surface antigen that has been developed as a tumor marker for clinical use, particularly as a marker of human breast cancer. The large portion of the core MUC-1 protein is made up of the tandem repeat domain of 20 amino acid units (N-PDTRPAPGSTAPPAHGVTSA-C). We have isolated hybridoma cells secreting Ab1 (AR20.5, antibody against MUC-1) and anti-idiotype antibody Ab2 (AR42.1, antibody against AR20.5) by the standard hybridoma-fusion technique. In particular, for the generation of AR42.1, antibody fragment Fab of AR20.5 was used to immunize Balb/c mice. The spleen cells were fused to mouse myeloma cell line NS0 (ATCC, Rockville, MD). The supernatants of growing hybridomas were screened against Fab of AR20.5 and cloned by limiting dilution. Clones of both AR20.5 and AR42.1 were found to be monoclonal and of isotype IgG1 kappa.

We postulated that AR42.1 might have a mimicry binding epitope as to antigen MUC-1. To test our hypothesis, a competition binding assay involving Ab2 (AR42.1) and the MUC-1 antigen was carried out, in which a fixed amount of biotinylated AR20.5 was reacted with different amounts of purified AR42.1 or control antibody MOPC-21 (Sigma) on ELISA. Microtiter plates (Maxisorp, Nunc) were coated with 100 μl of the MUC-1 epitope peptide (2.5 μg/ml; Biotool, Edmonton, Canada) overnight at 4°C. The wells were blocked with 1% BSA in PBS for 1 h at room temperature, and then reacted with a mixture of 50 μl of biotinylated AR20.5 (1,000 dilution) and 50 μl of AR42.1 or MOPC-21 for 1 h. After washing with 0.05% Tween 20 in PBS, avidin-HRP (100 μl) was added to the plates, followed by incubation for 30 min. The reactions were determined by adding 100 μl of a mixture of ABTS substrate and H_2O_2 in the ratio of 1:1 (KPL, USA), followed by reading with an ELISA reader at 405/490 nm. As shown in Fig. 1, the inhibition of the binding of AR20.5 to the MUC-1 peptide by AR42.1 was dose-dependent. AR42.1 at the concentration of 10 μg/ml inhibited AR20.5 binding to the MUC-1 peptide by 80%, whereas the control antibody did not cause significant inhibition. This indicated that AR42.1 shared the same binding epitope with MUC-1. Since glycosylation of MUC-1 that is expressed on the cell surface may alter the antibody recognition, it would be interesting to examine the binding using MUC-1 expressing cells in our future study.

The anti-idiotypic antibody Ab2 may have different specificities; some may recognize the antigen-binding site and express the internal image of the primary immunogen (Ab2β), while others may recognize sites present elsewhere in the variable region (Ab2γ and Ab2α) (*5*). To further demonstrate AR42.1 is Ab2β, we immunized Sprague-Dawley

[1] To whom correspondence should be addressed at the present address: K-165 Health Science Building, Department of Medicine, University of Washington, Seattle, WA 98195, USA. Tel: +1-206-543-5284, Fax: +1-425-486-8349, E-mail: dluo@u.washington.edu

female rats of 12 weeks of age to induce anti-AR42.1 antibodies (Ab3). After collection of pre-immune serum samples, rats received two intraperitonium immunizations of AR42.1 with a 1-week interval. Two hundred micrograms of AR42.1 was administered with 10 mg of microspheres as an adjuvant (*6*). Cardiac acupuncture was performed to obtain blood 2 weeks after the second immunization. The production of anti-AR42.1 antibodies (Ab3) was analyzed by ELISA. Briefly, microtiter plates were coated with the MUC-1 peptide and left at 4°C overnight, and then blocked with 3% BSA/PBS for 1 h and incubated with rat serum at different dilutions (1:100, 1:500, 1:1,000, and 1:5,000) for 1 h at room temperature. The enzymatic reactions were carried out by adding peroxidase conjugated goat anti-rat IgG (H+L) and ABTS substrate (KPL, USA). As shown in Fig. 2,

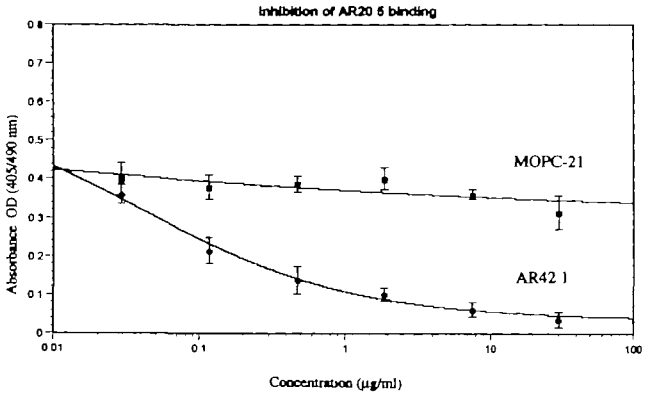

Fig. 1. **Inhibition of Ab1 (AR20.5) binding to the MUC-1 peptide by Ab2 (AR42.1) on ELISA.** The MUC-1 peptide (0.25 μg/well) was used to coat the plate, and the binding of biotinylated AR20.5 to the MUC-1 peptide was examined for inhibition in the presence of different amounts of AR42.1. An unrelated antibody, MOPC-21, was used as a negative control.

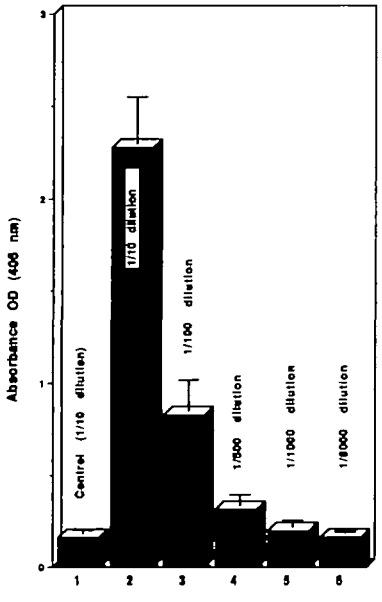

Fig. 2. **Determination of AR42.1 as AB2β by ELISA.** Plates were coated with the MUC-1 peptide and then incubated with rat serum at different dilutions (1:100, 1:500, 1:1,000, and 1:5,000). The absorbance was read at 405 nm.

AR42.1 induced Ab3, which recognizes the original antigen MUC-1, indicating that AR42.1 is Ab2β.

To obtain a better understanding of the antigen-Ab2 mimicry at the molecular level, we isolated the genes coding for variable heavy and light chains of AR20.5 (Ab1) and AR42.1 (Ab2), and compared the amino acid sequences of AR42.1 and the MUC-1 antigen. Briefly, total RNA was isolated from 1×10^6 hybridoma cells and then the first strand of cDNA was synthesized by reverse-transcription reaction using the $d(T)_{17}$ primer. The polymerase chain reaction (PCR) was carried out in a 100 μl reaction mixture containing 2 μg of RNA and 15 pmol of each primer (for Vl chain: Vk1FOR and Vk1BACK; for Vh chain: VH1FOR and VH1BACK) (*7*). The PCR amplification was performed with a typical cycle of denaturation at 94°C for 1 min, annealing at 30–42°C for 1.5 min, and elongation at 72°C for 1.5 min. The PCR products were gel-purified and ligated into cloning vector pBluscript KS (Stratagene) at the *Eco*RV site. The cloned DNA fragments were sequenced for both strands with an Applied Biosystems 373A DNA Sequencer (University of Alberta, Edmonton, Canada) using T3 and T7 primers. A comparison of the sequences of the Vl and Vh domains from AR20.5 (Ab1) and AR42.1 (Ab2) with the Kabat protein database (*8*) revealed six Complementary Determining Region (CDR) sequences for each antibody gene (Table I). Alignment of the MUC-1 antigen sequence with the CDR sequences of AR42.1 (Ab2) indicated that only its heavy chain CDR3 (H-CDR3) shows significant sequence homology to the MUC-1 repeating sequence, particularly in the PDTRP determinant motif of MUC-1.

MUC-1	Ala **Pro** Asp Thr **Arg Pro** Ala Pro **Gly** Ser
AR42.1 H-CDR3	Gly **Pro** Leu Tyr **Arg Pro** Gly Glu **Gly** Tyr
position	102

Beside the primary sequence homology, we further analyzed the molecular interaction of H-CDR3 of AR42.1 and MAb AR20.5 by means of structural homology modeling and computer docking experiments. The homologous proteins for Ab1 (AR20.5) and Ab2 (AR42.1) were obtained using on-line database search program LBAST (*9*), and their structures were chosen based on the sequence identity and the quality of the X-ray structure as the template molecules for the Vh domains of Ab1 and Ab2, respectively. The coordinate of the template molecule was obtained from the Protein Data Bank (*10*), and model structures of Ab1 and Ab2 were generated through the Whatif program (*11*). After sequence alignment, the initial crude model structures were subjected to extensive energy minimization using the X-PLOR program (*12*), and the candidates were further assessed for "correctness" using the PROCHECK program (*13*). Docking simulations between the Ab1 and

TABLE I. **Amino acid sequences of antibody CDRs.**

	Ab1 (AR20.5)	Ab2 (AR42 1)
L-CDR1	RSSQTIVHSNGKIYLE	RASSSINYMH
L-CDR2	RVSKRFS	ATSNLAS
L-CDR3	FQGSHVPWT	HQWSSSPRT
H-CDR1	NYWMN	ITSGYYWN
H-CDR2	EIRLKSNNFATHFAESVKG	YISFDGSNNYNPSLKN
H-CDR3	HYYGDY	GPLYRPGEGY

Note: Trp 125 of H-CDR1 (Ab1), Tyr214 and Tyr215 of H-CDR3 (Ab1), and Tyr100 and Pro102 of H-CDR3 (Ab2), which appear in Fig. 3, are highlighted in the table.

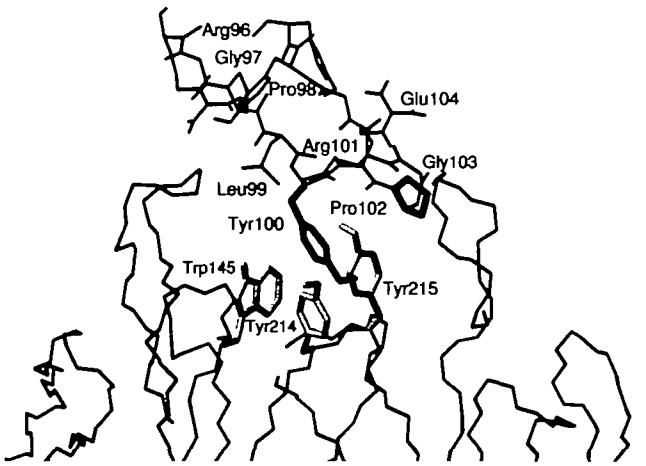

Fig. 3. **3D structural model of the Ab1/Ab2 complex.** In this model, an artificial scFv (single chain Fv with Vl-linker-Vh orientation) of Ab1 served as the rigid base for the interaction with Vh of Ab2. The hydrophobic CDR-H3 (Tyr214 and Tyr215) and CDR-H1 (Trp145) of Ab1 interacted with CDR-H3 (Tyr100 and Pro102) in Ab2.

Ab2 models were conducted using the DockVision program (*14*). Each of Vl and Vh of Ab2 was used as the target molecule, and an artificial scFv (single chain Fv with Vl-linker-Vh orientation) of Ab1 served as the rigid base of the binding receptor. Atomic interaction grids with a 0.5 Å step size in a 50-Å^3 box centered on the CDRs of Ab1 were used to define the search region for the target molecule. A hybrid Monte Carlo/simulated annealing algorithm was used to generate energetically favorable positions for the Ab2 ligand in the specified search region. To search as much of the possible conformational space as possible, 1,000 independent Monte Carlo searches were performed with 100 constant temperature cycles for simulated annealing. During the search, a Lennard-Jones 6-12 function, *i.e.* torsion angle terms together with a standard electrostatic function, was used in the force field. The top ten energetically favorable binding modes were chosen for further analysis. The 3D structural models of the Ab1 and Ab2 Vh domains were built. The PROCHECK results showed that these two model structures fulfilled the criteria for typical structures at 2.0 Å X-ray resolution. This favorable outcome reflected the high sequence identity between the model and template molecules (>80%) as well as the high structural quality of template molecules. The 3D structural model of Ab2 demonstrated the amino acids of interest at positions located in the hypervariable antigen binding loop of the heavy chain (H-CDR3, Fig. 3). The most stable binding model identified by evaluating the binding energy involving the atom-specific hydrophobic (*15*) and hydrogen bond interactions in the Monte Carlo simulations indicated that hydrophobic positions Tyr100 and Pro102 from H-CDR3 of Ab2 interacted with H-CDR1 at Trp145, and H-CDR3 at Tyr214 and Tyr215 of Ab1 (Fig. 3).

REFERENCES

1. Livingston, P. (1997) Immunotherapy of cancer with vaccines. *Encyclopedia Cancer II*, 916–926
2. Jerne, N.K. (1974) Towards a network theory of the immune system. *Ann. Immunol.* **125**, 373–389
3. Cleave, V.H., Van, Naeve, C.W., and Metzger, D.W. (1988) Do antibodies recognize amino acid side chains of protein antigens independently of the carbon backbone? *J. Exp. Med.* **167**, 1841–1848
4. Raychaudhuri, S., Kang, C.-Y., Kaveri, S.-V., Kieber-Emmons, T., and Koehler, H. (1990) Tumor idiotype vaccines. VII. Analysis and correlation of structural, idiotypic, and biologic properties of protective and nonprotective Ab2. *J. Immunol.* **145**, 760–767
5. Steinitz, M., Tamir, S., Froedin, J.-E., Lefvert, A.-K., and Mellstedt, H. (1988) Human monoclonal anti-idiotypic antibodies. I. Establishment of immortalized cell lines from a tumor patient treated with mouse monoclonal antibodies. *J. Immunol.* **141**, 3516–3522
6. Ma, J., Luo, D., Kwon, G.S., Samuel, J., Noujaim, A.A., and Madiyalakan, R. (1998) Use of encapsulated single chain antibody for induction of anti-idiotypic humoral and cellular immune responses. *J. Pharm. Sci.* **87**, 1375–1378
7. Orlandi, R., Guessow, D.H., Jones, P.T., and Winter, G. (1989) Cloning immunoglobulin variable domains for expression by the polymerase chain reaction. *Proc. Natl. Acad. Sci. USA* **86**, 3833–3837
8. Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry H.M., Gottesman, K.S., and Foeller, C. (1991) *Sequence of Proteins of Immunological Interest*, 5th ed., NIH publication No. 91–3242, U.S. Department of Health and Human Services, Bethesda
9. Madden, T.L., Tatusov, R.L. and Zhang, J. (1996) Applications of network BLAST server. *Methods Enzymol.* **266**, 131–141
10. Bernstein, F.C., Koetzle, T.F., Williams, G.J.B., Meyer, E.F., Brice, M.D., Rogers, J.R., Kennard, O., Simanouchi, T., and Tasumi, M.J. (1977) The Protein Data Bank: a computer-based archival file for macromolecular structures. *J. Mol. Biol.* **112**, 535–542
11. Vriend, G. (1990) A molecular modeling and drug design program. *J. Mol. Graph.* **8**, 52–56
12. Brunger, A.T. (1992) *X-PLOR 3.1 Manual*, Yale University, New Haven, CT
13. Laskowski, R.A., Macarthur, M.W., Moss D.S., and Thorton, J.M. (1993) Main-chain bond lengths and bond angles in protein structures. *J. Appl. Crystallogr.* **26**, 283–291
14. Trevor N.H., Stever, R.N., and Randy, J.R. (1997) *Proteins Struct. Funct. Gene. Suppl.* **1**, 205–209
15. Eiserberg, D. and McLachlan, A.D. (1986) Solvation energy in protein folding and binding. *Nature* **319**, 199–203