Vol. 164, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1989 Pages 271-276

A NOVEL STRATEGY FOR PRODUCING CHIMERIC BISPECIFIC ANTIBODIES BY GENE TRANSFECTION

Sirirurg Songsivilai, Patricia M. Clissold, and Peter J. Lachmann

MRC Molecular Immunopathology Unit, Medical Research Council Centre and University of Cambridge Clinical School, Hills Road, Cambridge CB2 2QH, England

Received September 4, 1989

SUMMARY: We have used genetic manipulation to produce chimeric bispecific antibodies. Plasmids containing variable regions of immunoglobulin from a murine hybridoma secreting anti-hepatitis B surface antigen were joined to human constant regions. These chimeric plasmids were introduced into transfectomas, secreting chimeric antibodies to iodo-hydroxy-nitrophenyl, by electroporation. Transfectomas secreting bispecific antibodies were identified. This approach has advantages over the fusion of hybridomas or chemical linking of two antibody molecules and will enable the use of bispecific antibodies *in vivo*.

The use of bispecific monoclonal antibodies (BsAbs) for immunodiagnosis and therapy have shown some encouraging results. They have been used for targeting effector cells (1-3) and delivering effector substances such as toxins and drugs (4,5) to tumours. The production of murine BsAbs has concentrated mainly on two methods: fusion of two hybridomas or hybridoma and immune cells (6,7), and chemical linking of two antibody molecules or their derivatives (8,9).

For *in vivo* immunotherapy, human immunoglobulins would be preferred since the murine or rat monoclonal antibodies induce an anti-globulin response. In a recent development, murine antibodies have been genetically modified by replacing the constant regions with their human counterparts (10-12). These

Abbreviations used are : BsAbs, bispecific antibodies; HBsAg, hepatitis B surface antigen; NIP, 5-iodo-4-hydroxy-3-nitrophenyl, Ig, immunoglobulin; E, enhancer; P, promoter; K enh, IgKappa enhancer; gpt, xanthine-guanine phosphoribosyltransferase; hyg, hygromycin; amp, ampicillin.

0006-291X/89 $1.50

chimeric human antibodies have been shown to be less immunogenic than murine antibodies.

We describe here a new strategy for producing bispecific antibodies by introducing two sets of chimeric human immunoglobulin genes with different specificities into murine myeloma cells. Antibodies with both designed specificities were identified in the cell culture supernatants. This approach can be used to produce bispecific antibodies for *in vivo* immunodiagnosis and therapy, and will enable BsAbs to be made, that cannot be produced by conventional techniques.

MATERIALS AND METHODS

Plasmid construction. A murine hybridoma secreting anti-hepatitis B surface antigen (HBsAg), D2H5, was kindly given by Dr. R. S. Tedder, Middlesex Hospital, London, U.K. (13). Construction of plasmids was based on a modified procedure of Orlandi et al. (14). Briefly, mRNA was prepared using oligo(dT)-cellulose columm (15). Double-stranded cDNA synthesis was performed using a cDNA synthesis kit (Pharmacia LKB Biotechnology, Uppsala, Sweden). Variable regions of heavy and light chains of immunoglobulin were amplified by polymerase chain reaction using primers derived from the relatively conserved sequences at each variable region end, and were then force-cloned into M13-VHPCR1 and M13-VKPCR1 vectors for sequencing. Nucleotide sequences were determined by the dideoxynucleotide sequencing method (16). The amplified variable regions were recloned into plasmids derived from pSV-gpt (aLys-30) and pSV-hyg (aLys-17) (kindly provided by Dr. J. Foote, Laboratory of Molecular Biology, Cambridge, U.K.) containing human immunoglobulin constant regions, γ1 and kappa respectively.

Antibody expression. Linearised plasmids were cotransfected by electroporation into a non-secreting murine myeloma cell line NS0 (17) and into transfectomas, THG1-24 and JW183/5/1, secreting chimeric human antibodies (IgG1, and IgG2 respectively, kindly provided by Dr. M. Bruggemann, AFRC Institute of Animal Physiology and Genetics, Babraham, U.K. (18)), to 5-iodo-4-hydroxy-3-nitrophenyl (NIP). Cells were selected in the presence of mycophenolic acid and hygromycin. Cell culture supernatants were collected seven days after transfection.

Assay for anti-HBsAg. Anti-HBsAg antibodies were determined by AUSAB radioimmunoassay kit (Abbott Labs., IL, USA). Results are expressed as means and standard deviations of radioactivity of duplicated values.

Assay for Bispecific antibodies. Bispecific anti-HBsAg/anti-NIP antibody activity was assayed by two radioimmunoassays. Briefly, a microtitre plate was coated with NIP-albumin, incubated with cell culture supernatants, washed, and then incubated with [^{125}I]recombinant HBsAg (kindly provided by Professor K. Murray, Department of Molecular Biology, University of Edinburgh, U. K.). In the reciprocal assay, HBsAg-coated beads (Abbott Labs, IL, USA) were used in combination with [^{125}I]NIP-albumin. Results are expressed as means and standard deviations of radioactivity of duplicated values.

RESULTS

Structures of the expression plasmids, pSV-gpt-H-D2H5 and pSV-hyg-L-D2H5, containing the variable regions of murine anti-HBsAg antibody, D2H5, assembled together with human heavy (γ1) and light (κ) constant regions respectively, are shown in figure 1. Mouse IgH enhancer (E) and promotor (P) are located upstream of the variable regions. In addition, the IgKappa enhancer is located between the variable and constant region exons of pSV-hyg-L-D2H5 (J. Foote, personal communication). Nucleotide and derived amino acid sequences of variable region of D2H5 were compared with the consensus sequences of immunoglobulin genes in the Kabat database (19) and were identified as members of family IIIc and family III of the murine heavy and kappa genes respectively (data not shown).

NS0 cells transfected with these plasmids secreted antibodies with anti-HBsAg specificity (Figure 2). After being transfected with the plasmids containing anti-HBsAg activity, bispecific anti-HBsAg/anti-NIP antibodies were identified in cell culture supernatants from THG1-24 and JW183/5/1 cells, using both radioimmunoassays (Figure 3).

DISCUSSION

Bispecific antibodies produced by chemically linking antibody molecules or their derivatives have different physical and

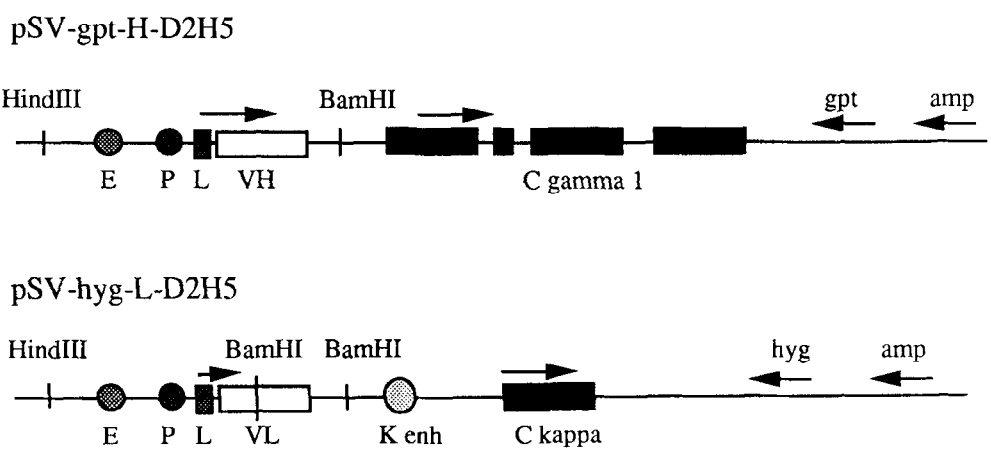

Figure 1. Schematic diagrams (not drawn to scale) of the expression plasmids pSV-gpt-H-D2H5 and pSV-hyg-L-D2H5, containing the variable regions of heavy and light chain of D2H5 (open box) joined to the human gamma1 and kappa constant regions (close box) respectively. Immunoglobulin enhancer (E) and promoter (P) are shown. pSV-hyg-L-D2H5 has an additional IgKappa enhancer (K enh) between the variable and heavy region exons. Drug resistance markers, guanine-xanthine phosphoribosyltransferase (gpt), hygromycin (hyg), and ampicillin (amp) are also shown.

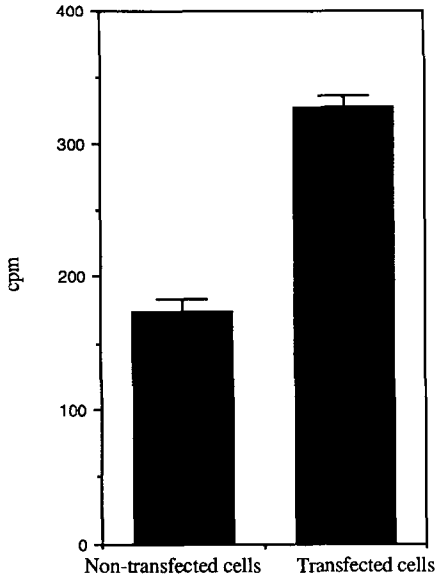

Figure 2. Assay for anti-HBsAg activity in supernatants (7th day after transfection) of NS0 cells transfected with plasmids pSV-gpt-H-D2H5 and pSV-hyg-L-D2H5 comparing with the non-transfected cells. Results are expressed as means and standard deviations of duplicated values.

biological properties from native immunoglobulin molecules. Chemical manipulations frequently disturb the biological activity of antibody by alteration of the antigen-binding sites (5,6).

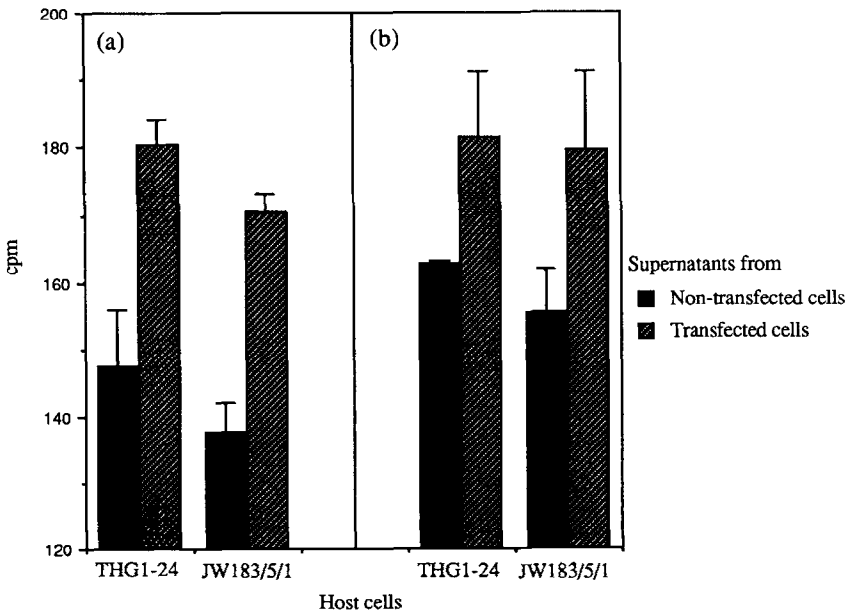

Figure 3. Assays for bispecific antibody activity using a NIP-coated plate (a), and HBsAg-coated beads (b) (see text). Host cells, THG1-24 and JW183/5/1, are transfectomas secreting chimeric human anti-NIP antibodies (IgG1 and IgG2 respectively).

Heteroconjugates may also suffer from limited access to tumours due to their size. Their stability *in vitro* and *in vivo*, and pharmacokinetic has yet to be investigated.

Hybrid hybridomas obtained from fusion of two hybridomas secrete two sets of heavy and light chains. By total random association of the two heavy and light chains, ten different combinations of immunoglobulin molecule are generated, only one has the desired bispecific activity (20). Chromosomes of these hybridomas are polyploid and unstable (21). Yield of bispecific antibodies also depends on the affinity between heavy chains from both parents and, theoretically, should be lower than expected from random association since the hybrid molecule containing different heavy chains should be less stable than the native one (6). In addition, the total absence of association between the two heavy chains of different class or subclass, such as between IgM or IgA and IgG (7,22), may prevent the formation of bispecific molecules.

Here we show the production of bispecific antibodies by genetic manipulation. Variable regions have been amplified from cDNA by polymerase chain reaction and inserted into a set of specifically engineered vectors to facilitate cloning (14). They were recloned into plasmids containing human constant regions that have been chosen for the best match between the two heavy chains. This allows random heavy chain association for the best yield of bispecific antibody. Since only the variable regions are derived from the parent hybridomas, BsAbs that cannot be produced due to the parental heavy chain incompatibility can also be engineered by replacing with the heavy chains of suitable class or subclass. In addition, for some applications where polyvalent, rather than monovalent, bispecific antibodies are preferred (7), dimeric IgA or pentameric IgM bispecific or multispecific monoclonal antibodies may be constructed by selecting the appropriate heavy chains.

Since bispecific antibody specificity is obtained by introducing only the small chimeric genes and drug resistance markers which will be integrated into the host genome, transfectomas will have smaller number and complexity of chromosomes compared with hybrid hybridomas from cell fusion, and, thus, should be more stable.

The strategy described here will provide a very powerful and flexible tool for the production of the more useful bispecific antibodies for immunodiagnosis and therapy.

ACKNOWLEDGMENTS

We are grateful to Dr. G. Winter for oligonucleotide primers, cloning vectors, and for his invaluable critical discussion. We would also like to thank Drs. M. G. Wing, D. H. Gussow, P. T. Jones, and B. A. Fernie for their helpful advice, Drs. R. S. Tedder, M. Bruggemann, J. Foote, and Professor K. Murray for some materials used in this work.

Dr. S. Songsivilai is supported by Anandhamahidol Foundation of Thailand and the Overseas Research Student Award.

REFERENCES

1. Staerz, U. D., Kanagawa, O., and Bevan, M. J. (1985) Nature 314, 628-631.
2. Liu, M. A., Kranz, D. M., Kurnick, J. T., Boyle, L. A., Levy, R., and Eisen, H. N. (1985) Proc. Natl. Acad. Sci. USA 82, 8648-8652.
3. Lanzavecchia, A., and Scheidegger, D. (1987) Eur. J. Immunol. 17, 105-111.
4. Corvalan, J. R. F., Smith, W., Gore, V. A., and Brandon, D. R. (1987) Cancer Immunol. Immunother. 24, 133-137.
5. Webb, K. S., Ware, J. L., Parks, S. F., Walther, P. J., and Paulson, D. F. (1985) Cancer Treat. Rep. 69, 663-672.
6. Milstein, C., and Cuello, A. C. (1983) Nature 305, 537-540.
7. Urnovitz, H. B., Chang, Y., Scott, M., Fleischman, J., and Lynch, R. G. (1988) J. Immunol. 140, 558-563.
8. Karpovsky, B., Titus, J. A., Stephany, D. A., and Segal, D. M. (1984) J. Exp. Med. 160, 1686-1701.
9. Glennie, M. J., Brennand, D. M., Bryden, F., McBride, H. M., Stirpe, F., Worth, A. T., and Stevenson, G. T. (1988) J. Immunol. 141, 3662-3670.
10. Morrison, S. L., Johnson, M. J., Herzenberg, L. A., and Oi, V. T. (1984) Proc. Natl. Acad. Sci. USA 81, 6851-6855.
11. Boulianne, G. L., Hozumi, N., and Shulman, M. J. (1984) Nature 312, 643-646.
12. Neuberger, M. S., Williams, G. T., Mitchell, E. B., Jouhal, S. S., Flanagan, J. G., and Rabbitts, T. H. (1985) Nature 314, 268-270.
13. Tedder, R. S., Guarascio, P., Yao, J. L., Lord, R. B., Eddleston, A. L. W. F. (1983) J. Hyg. Camb. 90, 135-142.
14. Orlandi, R., Gussow, D. H., Jones, P. T., Winter, G. (1989) Proc. Natl. Acad. Sci. USA 86, 3833-3837.
15. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning, A Laboratory Manual, pp. 188-209, Cold Spring Harbour, New York.
16. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.
17. Kearney, J. F., Radbruch, A., Liesegang, B., and Rajewsky, K. (1979) J. Immunol. 123, 1548-1550.
18. Bruggemann, M., Williams, G. T., Bindon, C. I., Clark, M. R., Walker, M. R., Jefferis, R., Waldmann, H., and Neuberger, M. S. (1987) J. Exp. Med. 166, 1351-1361.
19. Kabat, E. A., Wu, T. T., Reid-Miller, M., Perry, H. M., and Gottesmann, K. S. (1987) Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services, U.S. Government Printing Service.
20. Suresh, M. R., Cuello, A. C., and Milstein, C. (1986) Methods Enzymol. 121, 210-228.
21. Galfre, G., and Milstein, C. (1981) Methods Enzymol. 73, 3-46.
22. Zimmerman, B., and Grey, H. M. (1971) J. Immunol. 107, 1788-1790.