

## BISPECIFIC ANTIBODY DETERMINANTS

The IgG antibodies are known to consist of two half-molecules, each consisting of a light (L) chain and a heavy (H) chain. The H chains of the two halves are linked by disulfide bonds, which can be broken by selective reduction. If this step is performed for two different IgG samples, the half-molecules can be combined to form hybrid antibodies. This has been accomplished using intact rabbit globulins; Nisonoff et al. (1964) Science 134, 376-379.

Hybrids have also been formed using the F(ab')<sub>2</sub> fragments of IgG antibodies, rather than intact antibodies; i.e., the F(c) portions of the molecules, which do not provide immunospecificity, are, prior to hybridization, removed by digestion with an appropriate protease such as papain. This procedure has been described in Nisonoff et al. (1960) Arch. Biochem. Biophys. 89, 230-244 and in Nisonoff and Rivers (1960) Arch. Biochem. Biophys. 93, 460-462. In a later discussion of the first paper Nisonoff wrote, in Current Contents (Nov. 2, 1981) 44, 25:

So far this procedure has had limited application, principally in the staining of cell surfaces with ferritin by using a hybrid of anti-ferritin antibody and antibody to a cell surface antigen. The use of hybrid antibody has also been considered as a means of bringing a pharmacological agent specifically into contact with a desired tissue surface.

The use of such hybrids for the delivery of cytotoxic drugs has also been suggested in Raso and Griffin (1978) Fed. Proc. 37, 1350.

Milstein (1981) Proc. R. Soc. Lond. B211, 393-412 suggests the possibility of using "monoclonal antibodies as carriers of toxic substances for specific treatment of tumors," and states that "(i)t is possible that Fab fragments will be better targeting agents than intact antibody."

Hybrid antibodies have also been formed by fusing two cells, each capable of producing different antibodies, to make a hybrid cell capable of producing hybrid antibodies. Such a method is described in Schwaber et al. (1974) P.N.A.S. U.S.A. 71, 2203-2207, Mouse myeloma cells were fused to human lymphocytes, and the resultant fused cells produced "hybrid antibody molecules containing components of mouse immunoglobulins assembled with human heavy and light chains." The human antibody component was not monoclonal, and was undefined.

Schwaber et al. also describes an in vitro experiment in which the mouse and human antibodies were reduced strongly enough to break bonds between L and H chains, and then "allowed to recombine randomly."

In Cotton et al. (1973) Nature 244, 42-43 there is described an experiment in which mouse myeloma cells were fused to rat tumor cells to produce fusions which produced "an extra component" which was "likely . . . a hybrid mouse-rat light chain dimer" as well as "non-symmetrical molecules made up of one light chain of each parental type."

Another paper, Raso et al. (1981) Cancer Research 41, 2073-2078, describes the formation of an impure sample of rabbit antibody F(ab')<sub>2</sub> fragments against human IgG F(ab')<sub>2</sub> fragments; the rabbit antibody fragments were split by reduction and reassembled with antiricin A chain F(ab') fragments. The dual specificity

dimers were used in targeted drug delivery experiments. The article states:

The 2 types of purified antibodies used for this work were isolated from conventional heteroantisera. Thus, a complicated array of affinity and specificity combination must arise upon annealing these 2 populations. The advent of homogeneous hybridoma-derived antibodies will afford absolute control over the binding affinities of the constituent halves of a hybrid antibody, and this uniformity should greatly boost their ultimate effectiveness as delivery vehicles.

The present invention provides a homogeneous sample of identical bispecific antibody determinants, each bispecific determinant being composed of two L-H half molecules linked by disulfide bonds, each L-H half molecule being different from the other and being specific for a different antigenic determinant, and being composed of at least the F(ab') portion of a monoclonal IgG antibody.

The bispecific antibody determinants of the invention are made according to the following procedure. Using conventional methods, two different monoclonal IgG antibody samples are produced, each antibody having one of two desired specificities. If desired, each sample is then exposed to an appropriate protease such as papain to cleave off the F(c) portion of the antibody molecules to produce F(ab')<sub>2</sub> fragments. Each sample is then subjected to conditions sufficient to break at least some of the disulfide bonds linking the L-H half-molecules so that at least some of the antibodies are split into two half-molecules.

The two samples are then combined under conditions which permit at least some half-molecules of each determinant to chemically combine with at least some half-molecules of the other determinant to form the bispecific antibody determinants of the invention.

The bispecific antibody determinants molecules are then separated from the rest of the mixture. One separation method is contacting the mixture with an affinity matrix containing an antigen capable of specifically binding to either of the two halves of the bispecific antibody determinant, then eluting bound matrix-bound material, and contacting that material with an affinity matrix containing an antigen capable of specifically binding the other half-molecule. The material bound to this second matrix has the required dual specificity.

An alternative separation method can be used in a case where one of the halves of the bispecific antibody determinant has a specificity for an antigenic determinant which is a macromolecule (a molecule having a molecular weight greater than about 1000 daltons). This method involves adding the macromolecular antigenic determinant to the sample containing the bispecific antibody determinant to be purified to form immune complexes which can be separated into subfractions having different molecular weights by, e.g., gel filtration or electrophoresis. The subfraction having a molecular weight equivalent to the molecular weight of the complex of the desired bispecific antibody determinant with the macromolecular antigen is separated from the other subfractions, and, if desired, the macromolecular antigen is then removed using conventional methods.

In the drawings,

FIG. 1 is a diagrammatic representation of two different antigenic determinants linked by a bispecific antibody determinant.