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(71) Applicant: THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY
Encina 6-930, Stanford University
Stanford, California 94305(US)

(72) Inventor: Kaplan, Henry S.
631 Cabrillo
Stanford California, 94305(US)

(72) Inventor: Olsson, Lennart
20A Ibstrupvej
DK-2820 Gentofte(DK)

(74) Representative: Harrison, David Christopher et al,
MEWBURN ELLIS & CO 70 & 72 Chancery Lane
London WC2A 1AD(GB)

(54) **Human hybridomas, precursors and products.**

(57) Human monoclonal antibody compositions, human-human monoclonal hybridoma cells, human non-viral transformed particularly non-Epstein-Barr virus transformed, neoplastic lymphoid cells, human antibody genes and their uses. Human neoplastic cells are developed for fusing with immunized lymphoid cells to provide stable human-human hybridoma strains producing complete monoclonal antibodies for a predefined antigen. From a myeloma cell line, rapidly growing 8-azaguanine resistant HAT sensitive cells are selected. The selected myeloma cells are crossed with immunized lymphoid cells and the resulting cell mixture grown under controlled selective conditions. Lymphoma cells may be substituted for the myeloma cells. After expansion of the desired hybridoma cells, the monoclonal antibodies may be harvested. The hybridomas serve as a source for messenger RNA for light and heavy chains which may be used for production of light and heavy chain immunoglobulin proteins through hybrid DNA techniques.

U-266-AR$_1$ cell line has been deposited at Cell Distribution Center, The Salk Institute on July 17, 1980, and the A.T.C.C. on September 11, 1980.

EP 0 044 722 A1

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HUMAN HYBRIDOMAS, PRECURSORS AND PRODUCTS

The mammalian capacity for producing immunoglobulins has found application in medicine and industry. The ability of immunoglobulins to distinguish specifically between chemical compounds of slightly differing structure has found broad application in the detection and measurement of a wide variety of compounds. In therapeutic applications, immunoglobulins can be administered to provide passive immunity against diseases. Major stumbling blocks in the wide application of immunoglobulin therapy were the heterogeneity of antisera and the limited availability of human antisera for a specific antigen.

The seminal discovery by Kohler and Milstein of mouse "hybridomas" capable of secreting specific monoclonal antibodies against predefined antigens ushered in a new era in experimental immunology. Many of the problems associated with heteroantisera are circumvented; the clonal selection and immortality of such hybridoma cell lines assure the monoclonality, monospecificity and permanent availability of their antibody products. At the clinical level, the use of such antibodies is clearly limited by the fact that they are foreign proteins and would act as antigens to humans.

Human cells have only been difficultly cultured *in vitro*. Efforts to achieve a human hybridoma which is a cross between a lymphoid cell and a myeloma cell have heretofore

been unsuccessful. The problems of maintaining a stable culture of human cells have inhibited the ready production of human-human hybridomas.

The production of mouse hybridomas is described by Kohler, G. and Milstein, K. (1975) Nature 356: 495-7; (1976) Euro. J. Immunol 6: 511-519. Chimeric hybridomas generated by fusing mouse myeloma cells with human immunoglobulin-producing cells were described by Levy, R. and Dilley, I. (1978) PNAS USA 75: 4211-2415. Permanent cultures of specific antibody-producing human B-lymphocytes obtained by transformation with Epstein-Barr virus is described by Steinitz, M. (1977) Nature 269: 420-422.

SUMMARY OF THE INVENTION

Non-viral transformed, particularly non-Epstein-Barr virus transformed, neoplastic lymphoid cells are grown under conditions to provide strains having HAT sensitivity for use as fusion partners. The neoplastic lymphoid cells may then be fused with lymphocytes to provide hybridomas capable of stably producing immunoglobulins to a predetermined ligand.

In accordance with the subject invention, novel human neoplastic lymphoid cell strains are provided, which are employed for fusion with lymphoid cells to produce hybridomas capable of producing complete monoclonal antibodies having a unique specificity and homogeneous composition. The invention therefore involves the development of the neoplastic lymphoid cell strains; the preparation of lymphoid cells producing antibodies to a specific antigen; the fusion of the immunized lymphoid cells and neoplastic lymphoid cells to produce hybridoma cells; the selective culturing of the hybridoma cells; and the production of monoclonal antibodies. The antibodies may be produced to a wide variety of haptens and antigens and may find use in immunoassays, passive immunization, treatment against infection, diagnosis and treatment of cancer, and the like. In addition to the production of IgG, human-human hybridomas

offer opportunities for the production of complete human monoclonal IgA, IgM, and IgE.

The human-human hybridomas can also serve as a useful source of mRNA for the heavy and light chains of antibodies for specific antigens. By known molecular biology techniques, the mRNAs may be used for the generation of genes which when inserted into the appropriate vector can serve as a source of the proteins. Upon assembling of the light and heavy chains, antibodies are produced.

Non-viral transformed, particularly non-Epstein-Barr virus transformed, neoplastic lymphoid cells may be employed as fusion partners. The fusion partners are characterized by being differentiated, HAT medium sensitive and unable to metabolize hyperxanthine. Illustrative of neoplastic lymphoid cells are cells obtained from a host with a lymphoma and cells obtained from a host with a myeloma. The lymphocytes are the principal cell type of lymph tissue.

Human Lymphoma Cell Line.

A human lymphoma may be modified as described below for a myeloma line to provide a HAT sensitive fusion partner. The lymphoma line may then be employed in the same way as the myeloma line to provide hybridomas for the production of immunoglobulins specific for a predetermined determinant.

Human Myeloma Cell Line

The human myeloma cell line is chosen to provide a stable cell line which is HAT medium sensitive and unable to metabolize hypoxanthine. The particular cell line chosen was U-266 which was originally described by Nilsson, K. et al., (1970) Clin. Exp. Immunol 7: 477-489.

HAT sensitivity is achieved by culturing cells in a medium containing a purine analog such as 8-azaguanine. Cells remaining viable under these conditions are mutants lacking an alternative biosynthetic pathway for the production of purines.

Specifically, the cells are first cultured at a high 8-azaguanine concentration, then at a low 8-azaguanine concentration, followed by cultivation at intermediate concentration levels. In each instance, incubation times are

about one week, with the viable cells being isolated prior to the next incubation. The 8-azaguanine concentration varies in the range of about 3 to 25µg/ml, usually in the range of about 5 to 20µg/ml. At each stage the number of cells being incubated should be sufficient to ensure the isolation of viable cells at the end of the incubation. There should be at least 1×10^3, preferably 5×10^3 cells per microwell. Alternatively, a sngle stage may be employed with a semisolid medium e.g. agarose.

The number of successive incubations with nutrient media containing 8-azaguanine will be at least two and not more than about eight.

Selection is further made of the fastest growing 8-azaguanine resistant HAT sensitive clones and it is these clones that are expanded. Rapidly growing clones normally double in about 24 to 36 hours.

Except for the 8-azaguanine and HAT, the nutrient media employed are conventional. Prior to fusion the selected cells are expanded in non-selective nutrient medium to enhance the number of cells.

Human Lymphoid Cells

The human lymphoid cells are cells immunized against a hapten or antigen. Various sources of lymphoid cells may be employed. One source is spleen specimens, which specimens are devoid of malignancies. The host should be immunized at least once, and at least about two weeks prior to the splenectomy. After freeing a single cell suspension of the spleen tissue of red blood cells and granulocytes, the viable mononuclear cells are suspended in an appropriate nutrient medium, and non-adherent cells separated from adherent cells. Desirably, the cells are grown in the presence of a mitogen for about 5-7 days to enhance fusibility. The lymphoid cell culture may then be fused with the myeloma cell line.

Instead of In vivo immunization, spleen cells can be isolated and immunized in vitro. A single cell suspension of spleen cells is prepared, viable cells are isolated and seeded in nutrient medium with the appropriate antigen at an appropriate concentration. After sufficient time for immunization, viable cells are isolated and used for fusion.

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