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Perspectives and Commentaries

Human Monoclonal Antibodies: New Approaches and Perspectives in Cancer

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THE SEARCH for specific tumor antigens has been actively pursued in recent years and yet an ideal antigen is difficult to obtain. In fact, even if we cannot detect an antigen on normal cells, it does not mean that a tumor-specific antigen is not present. On the other hand, the presence of an increased amount of a normal antigen may be more important than the appearance of a new antigen. Indeed, the abnormal amount of a normal antigen may reflect the distinction between normal and neoplastic cells (tumor-associated antigen). The origin of a tumor antigen can be endogenous or exogenous. The endogenous antigen is a product of a normal gene abnormally expressed: this category includes the oncofetal antigens which are normal proteins with some function in the fetus but not expressed at the same level in adults.

This antigen could also be the expression of genetic rearrangement during somatic division, leading to the removal of control elements in the DNA sequence or to the loss of an essential enzyme and the expression of new molecules.

By exogenous genetic material, we mean viral information coding for membrane proteins. The virus could carry various materials and the insertion of viral genome can lead to the expression of a viral protein or to the separation of a control element from its structural target. With the development of monoclonal antibodies (MoAbs) technology [1] a new step in tumor immunology has been reached, and in this paper we want to review new approaches in human MoAbs production and to assess whether they are superior to murine MoAbs in the recognition of tumor antigen.

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MURINE MONOCLONAL ANTIBODIES

The hybridization technique, first described by Kohler and Milstein for producing MoAbs, has recently been used for the diagnosis, localization and treatment of cancer [2–6]. The clonal selection of hybridoma cell lines ensures the monoclonability and the specificity of their antibody products and obviates the problems associated with the use of antisera.

The principal use envisaged for MoAbs was the recognition of neoantigen on tumor cells which had proven so difficult with serological methods; however, to date, not a single MoAbs has been identified as a 'pan tumor cell' reagent. Nevertheless, they have been very useful in many situations in cancer medicine [7], for instance: (1) tumor diagnosis by detection of tumor-associated antigens; (2) tumor localization using labelled antibodies; (3) detection and monitoring of hormones and other antigens in serum; and (4) targeting therapeutic agents to the tumor by the use of coupled antibodies.

With regard to the last point, the production of targeted cytotoxic reagents that would specifically kill the tumor in the tissue without harming normal cells represents another attractive goal for MoAbs [8, 9]. At present the dissociation of the toxic material (radioisotope, toxin) before reaching the tumor remains the major problem,

preventing a routine use of targeted cytotoxic reagents.

Ultimately, for the detection of micrometastases of tumor cells, it is well demonstrated that the use of a mixture of labelled antibodies to cover a broader range of antigen specificities has considerably improved the imaging performances [10], and we can speculate that this approach will be successful in the killing of tumor cells as well.

HUMAN vs MURINE MONOCLONAL ANTIBODIES

Although murine and other rodent antibodies are powerful new reagents in laboratory investigations, their clinical use is likely to be severely limited by the fact that they are foreign proteins capable of inducing immune responses. Therefore the production of human MoAbs became an attractive new goal to pursue. However, the production of human MoAbs is still at its beginning and it is somewhat premature to attempt to make any detailed comparison of the potential of human vs murine MoAbs at this time.

Nevertheless, a few important points should be mentioned. It seems likely that murine and human MoAbs will be equally useful for *in vitro* diagnosis. Moreover, murine MoAbs are likely to be preferable for practical and economical reasons. However, until now murine MoAbs have failed to detect certain major histocompatibility antigens or rhesus antigens which can be detected by human antibodies [11]. Therefore the murine model cannot reproduce the spectrum of human immune response.

It is clear that the major importance of human MoAbs is related to the fact that they can be administered safely and repeatedly in humans and thus may find applications in the diagnosis and/or treatment of cancer. The fact that each MoAB bears a single idiotype raises the possibility of generating anti-idiotype antibodies in response to the administration of a MoAb. This possible reaction encourages the production of multiple antibodies directed against the same antigen or against different antigenic determinants. Also, it will be better to use antibodies against antigens that are not shed into the blood stream in order to minimize the problem of immune complex formation and the associated potential toxicity.

HUMAN MONOCLONAL ANTIBODIES: PERSPECTIVES

In the last few years several methods have been developed to generate cell lines producing human MoAbs. Somatic cell hybridization between specific B lymphocytes and mouse myeloma cells have been hampered by the difficulty of establishing stable cell lines in culture, mostly due to the selective loss of human chromosomes. Sensitized B lymphocytes can be immortalized *in vitro* into B lymphoblastoid cell lines with Epstein-Barr virus (EBV). As reported by Katano *et al.*, this method has been successfully applied to the production of a human (IgM) MoAb against a tumor-associated antigen (OFA-1-2) [12]. The anti-OFA-1-2 Ab specifically reacts with malignant cells of neuroectodermal origin, including melanoma, glioma and neuroblastoma, and is highly cytotoxic to tumor cells *in vitro* in the presence of complement. The antibody is also cytotoxic to melanoma cells inoculated subcutaneously into athymic nude mice.

However, lymphoblastoid cell lines usually secrete IgM antibodies and since the distribution of IgM immunoglobulins seems to be restricted to the intravascular space, this antibody has to be injected into tumor nodules to suppress the tumor growth. This is illustrated by Katano's experiment in which intraperitoneal injection of the anti-OFA-1-2 antibody failed to alter the growth of the subcutaneously inoculated melanoma cells while the simultaneous direct subcutaneous injection did inhibit the growth of tumor cells.

Kaplan and Olsson were the first authors to describe a true human-human hybridoma secreting human MoAbs of predefined antigenic specificity, although the level of antibody production was too low for practical applications [13].

Human immunoglobulins resulting from the fusion between B lymphocytes and established lymphoblastoid cell lines (rather than myeloma cell lines) is a promising new method; a few human MoAbs against glioma, lung and vulvar carcinomas have been obtained using this procedure. However, the level of antibody production is still low and the poor cloning efficacy remains a problem [14].

Recently we described a new approach which combines EBV transformation and fusion with a human-mouse heteromyeloma cell line (SHM-D33). Following the fusion, the ouabain resistance of the SHM-D33 cell line allows the selection of the hybrids and the yield of immunoglobulin-producing hybrids is consistently high [11, 15].

The presence of circulating antibodies which react with autologous and allogenic tumors has been demonstrated in the serum of several cancer patients, supporting the speculation that some patients are naturally sensitized against tumor antigens. Thus the use of B lymphocytes from those patients or from lymphoid tissue draining the sites of tumor is likely to be the optimal source to produce anti-tumor human MoAb in our system. Also, one of the primary concerns for the

therapeutic use of antibodies produced by cell lines containing EBV or retrovirus is the possibility of viral contamination. In our study, the reverse transcriptose test was negative, arguing against the production of a murine retrovirus by the hybrid cells. In addition, analysis by molecular hybridization using EBV-specific probes and immunofluorescent straining for Epstein-Barr nuclear antigen demonstrated that the majority of the hybrids had lost the EBV genome. This fact, added to the greatly augmented level of immunoglobulin production, lends a major advantage to our system in comparison to previous reports.

This method should permit the generation of human MoAbs against a broader spectrum of predefined antigens. However, since this method can only use the lymphocytes from donors naturally sensitized against an antigen, the development of optimal techniques for *in vitro* antigen priming of human lymphocytes is still necessary to have a human model similar to the murine systems.

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