NEWER TECHNIQUES IN MONOCLONAL ANTIBODY PRODUCTION IN RELATION TO TUMOUR TARGETTING

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ABSTRACT

Apart from murine monoclonal antibodies, human monoclonal antibodies are of significance in tumour targetting in human subjects. These may be derived from fusion with human lymphoblastoid cell lines or by Epstein Barr (EB) transformations of lymphocytes harvested from tumour patients. The monoclonal antibody may be of a class *e.g.* IgM, which it may be necessary to modify to obtain an IgG or a particular subtype of IgG. Further, Fab. $F(ab)_2$ and whole antibodies have different properties when used for RID and RIT. New concepts in this area are critically reviewed.

INTRODUCTION

FOLLOWING Kohler and Milstein's epoch-making report[1] on the creation of unlimited quantities of monoclonal antibodies in vitro from hybridoma cells there has been a knowledge explosion in the application of such monoclonal antibodies to medicine and biology and this has already led these pioneer workers to score the Nobel Prize. The production and testing of monoclonal antibodies have also undergone considerable evolution. Monoclonal antibodies are now available not only from the fusion of lymphocytes with mouse myelomas secreting their own immunoglobulins but also non-secreting mouse myelomas, rat myelomas and now in particular human myelomas, which have obvious advantages over heterologous species for in vivo work in human subjects.

Since chromosomal constitution of intraspecific human hybrids is much more stable, human × human hybridomas are more likely to be a useful source of specific human monoclonal antibodies[2].

In addition there have been reports on the direct transformation of lymphocytes[3], without hybridization with myeloma lines. Another interesting development is the harvesting of lymphocytes already producing antibodies from patients instead of immunizing animals to obtain the desired lymphocytes[4].

Epstein Barr Virus Immortalization:

Several years before the application of hybridoma technology to the production of human monoclonal antibodies, human lymphoid lines producing antibody with defined antigenic specificity were established by Epstein-Barr Virus (EBV), immortalization[5]. EBV is a lymphotropic herpes virus which transforms normal B-lymphocytes, making them capable of culture as established lines. Pre-selection of antigen-specific cells may facilitate the establishment of specific cell lines since even after immunization in vivo, only a small fraction of the B-lymphocytes produce the desired antibody.

Rosen *et al*[6] demonstrated that direct infection of purified human blood lymphocytes with EBV in vitro induced polyclonal secretion of immunoglobulin. Culture supernatants assayed by radioimmunoassay (RIA) contained a heterogenous immunoglobulin isotypes and antibodies directed against various randomly selected antigens. It became apparent that if monospecific B cells expressing EBV receptors could be immortalized *in vitro* by EBV and then triggered to produce antibodies, permanent lines from B-lymphocytes might be established, which are capable of producing specific antibodies against any desired antigen. The main limitations of the EBV technique are the low quantities of antibody produced ($< 1\ \mu g/ml$) and the relative instability (< 8 months) of these lines. However, one can restore high values for antibody production in these declining EBV lines by somatic cell hybridization with a plasmacytoma cell lines. Further, the hybrid cells can perhaps be passed on to nude mice depleted of natural killer cells, thus solving the problem of bulk production. The presence of either EBV or retrovirus in monoclonal antibody preparations intended for human use remains a limitation. Xenotropic retroviruses are released from many murine hybridomas or fusion partners and are known to be infectious for human cells[7]. This knowledge, however, has not prevented the experimental use of murine monoclonal antibodies in man[8–11].

The EBV used for human hybridoma work originates from the B95–8 marmoset cell line[12]. This transforms

human B-lymphocytes *in vitro* and the EBV nuclear antigen, EBNA, is expressed but the viral cycle is not completed. Although infectious virus is not released, the possibility of contaminating hybridoma supernatants with transforming viral DNA does exist at least in theory. However, virus and viral DNA can be inactivated or removed from antibody preparations which can be monitored by sensitive B-cell transformation tests and possibly by injection into marmosets, a species in which EBV is rapidly fatal. EBV carrying human hybridomas can also be given in humans by implanting within cell impermeable chambers[2]. Thus EBV +hybridoma technique is very flexible since the use of EBV (i) immortalizes the donor B cell for future use and repeated fusions; (ii) aids expansion of rare antigen-specific B cells in the peripheral blood prior to fusion; and (iii) increases hybridization frequencies over 10-fold. However, only 1 of 21 hybridomas obtained in this way has secreted antigen specific IgG; most (20/21) produce IgM[2].

The human monoclonal antibodies produced by the EBV, hybridoma or EBV-hybridoma technique are potential tools for the diagnosis and treatment of human diseases. However, before this potential is realized, two major problems await solution: (a) the bulk production of monoclonal antibodies by growth of hybridomas as ascites tumours in experimental animals, and (b) the presence of either EBV or C-type virus in monoclonal antibody preparations intended for human use.

Radiolabelling of antibodies:

Pressman and Kerghley first labelled antibodies with radioactive isotopes[13] and showed that the rabbit antibodies to rat kidney, could be labelled with I-131 and localised in the kidneys of living rats after intravenous administration. An extrapolation of these results suggested to these and other investigators[14, 15], that one could prepare similar antibodies to malignant tumours for tumour diagnosis (radioimmunodetection—RID), or to carry therapeutic dose of radioactivity (radioimmunotherapy—RIT) or other therapeutic agents directly to tumours even when widely metastasized[16].

A major breakthrough occurred when monoclonal tumour specific antibodies enter this field of RID giving relatively high target to non-target ratios compared to earlier preparations of labelled antibodies since monoclonal antibodies are 99% immunospecific. Radioiodine labelled monoclonal antibodies have already successfully located tumours in clinical subjects[17, 18].

F(ab), F(ab)$_2$ fragments:

The report of Wahl *et al*[19] demonstrated that in a specific human colonic carcinoma xenograft model, the F(ab)$_2$ bivalent antibody fragment of a distinct monoclonal antibody raised against carcinoembryonic antigen (CEA) is superior to intact antibody or the monovalent F(ab) preparations for tumour imaging with I-131, because of smaller non-target nonspecific reticuloendothelial uptake. Goldenberg[18, 20] reported that radiolabelled F(ab)$_2$ monoclonal anti-CEA IgG permit earlier imaging of CEA-containing tumours as compared with the corresponding intact antibody; the background subtraction technique could contribute to improved tumour definition. The use of an irrelevant immunoglobulin F(ab)$_2$ with a different label other than that of the specific antibody may have the potential for improved and more rapid tumour imaging. As Wahl *et al* emphasize, large doses of the bivalent antibody fragment preparation may provide better imaging results, especially with smaller tumours. One should be able to image tumours less than 5 g and acquire localization data on tumours less than 1 g in weight to evaluate RID capabilities of an antibody[21].

Factors affecting radioimmuno imaging:

The rate of clearance of antibody from the circulation and from non-target tissues together with the rate of uptake, amount of accretion, and residence time in tumours, will all influence tumour imaging at any particular time. Both monoclonal and polyclonal antibodies reach peak levels in tumours 24 to 48 hr after injection, decreasing thereafter[22, 23]. Methods that facilitate clearance of antibody without interfering with tumour uptake would result in improved tumour imaging. The use of antibody fragments, as described by Wahl *et al*[19], as well as in previous studies with monoclonal antibodies used in different animal model[24, 25] is a step in this direction. Another approach is the administration of liposome-entrapped second antibody to enhance clearance of circulating radiolabelled antibody, resulting in improved tumour imaging[26]. In Goldenberg's view, use of the second antibody (directed against the first bearing the radiolabel) without liposomes is at least equally effective[18].

Monoclonal and polyclonal antibodies:

The report by Wahl *et al*[19] discusses the advantages of the F(ab)$_2$ over F(ab) fragment or intact IgG for tumour imaging, but does not offer a comparison with

similar fragments of a conventional, affinity-purified anti-CEA IgG. "The possibility that a monoclonal antibody may target better than a polyclonal one may be based on the supposition that antibody against the targetable epitope(s) can be produced in higher quantities with monoclonal antibody. This suggests, however, that suitably prepared polyclonal antibodies should be equally effective as monoclonal antibodies for tumour targetting. In fact, the more restricted specificity of the monoclonal antibody could be a disadvantage if there is considerable heterogeneity in the expression in neoplasms of a particular epitope recognized by the monoclonal antibody" (Goldenberg)[18]. In one clinical study, monoclonal and polyclonal (affinity—purified) antibodies against CEA gave similar imaging results and tumour uptake based on a comparison in a limited number of patients[27].

Monoclonal antibody engineered cocktails

In the past, consideration has been given exclusively to the use of single antibody preparation for tumour imaging or therapy. It may however, be, that highly specific and restricted monoclonal antibody preparations are not as effective for localization or therapy as antibody mixtures. Such mixtures may be of antibodies directed against different determinants of the same antigen, such as with CEA, monoclonal antibodies[28], or against different antigens of the same tumour cells[29]. Antibody mixtures may provide increased uptake of radioactivity or toxins in tumours, "especially neoplasms with heterogenous cell populations with variable quantities of different epitopes of an antigen or different tumour antigens expressed". This may have the desired results of improved resolution and better tumour:nontumour ratio thus perhaps reducing the need for background subtraction techniques. Such antibody mixtures for tumour localization and therapy may be considered as "engineered polyclonal cocktail of antibodies", since different mixtures of epitope-restricted monoclonal antibodies are combined to produce more effective cancer detection and/or therapeutic agents. Indeed, this may well be the principal contribution of monoclonal antibody technology for tumour RID and antibody-mediated therapy—the development of the proper components of an engineered polyclonal cocktail.

Hybrid-hybridomas—bifunctional immunoglobulins:

The recent creation of hybrid-hybridomas by Milstein and Cuello[30] has opened a new vista of bifunctional immunoglobulins which may be useful in reducing the number of ingredients in the engineered polyclonal cocktail. Here the hybridoma secreting a specific immunoglobulin against a certain antigen, is now fused with a lymphocyte immunized against a second different antigen, resulting in a new hybrid (second generation) hybridoma which secretes bifunctional immunoglobulins with dual specificity against both the antigens of interest such as for intracellular targets-DNA epitope using attachment to the cell surface antigens to gain cellular entry. Bifunctional immunoglobulins can also target both fibrinogen in the tumour cocoon as well as more tumour specific cell surface/intracellular antigens[31]. Bispecific immunoglobulins may also refine the gross diagnosis as well as the Feulgen type staining in malignant and premalignant lesions[32].

Radioimmuno-detection is alongside nuclear magnetic resonance one of the newest and most challenging diagnostic modalities, with applications reaching beyond oncology. During its gestation for the last 30 years, improvements in antibody preparation and purification, tumour-marker identification, and advances in imaging instrumentation and antibody labelling have all contributed to a resurgence of interest in the use of radiolabelled antibodies for specifically disclosing tumour sites by means of external imaging methods[33, 34]. The advent of monoclonal antibodies with their exquisite specificity and capacity to recognise more tumour-distinct markers will represent a further contribution towards the establishment of RID as a standard detection method. But even at its current stage of development, RID with I-131 labelled antibodies against CEA, alpha-fetoprotein, human chorionic gonadotropin, prostatic acid phosphatase, and insulin has proved useful in the assessment of sites of tumour in previously diagnosed cancer patients, revealing neoplasms as small as 1.5–2.0 cm[20, 35–40]. "It is for these reasons that the corresponding advances to be expected in the labelling of antibodies with more suitable radionuclides for imaging, in the use of single photon remission computerized tomography (SPECT), and in the development of simpler and more specific background subtraction techniques, if needed, will undoubtedly contribute to the maturation of RID to a common role in the detection and staging of cancer".

However, in the early development of a potential new modality for cancer therapy, the first and critical question is that of toxicity of the administered agent. In relation to the use of the radiolabelled immunoglobulins (RIT) and in addition to the considerations of sterility, pyrogenicity and environmental control of the

patient, the exclusion of potentially hypersensitive patients (history of asthma, eczema, allergic reactions) and the minimization of nonspecific uptake is important.

It is hoped that monoclonal antibodies labelled with high specific activity may achieve a high tumour dose rate. Direct implantation of labelled antibody in tumour bearing sites, gives a radiation dose of 40–60 rads per hour, significantly greater than that achieved by intravenous injection, which gives 2–5 rads per hour. If we could achieve a dose rate of 20–40 rads per hour in the tumour bearing area, this would represent to radiotherapists a "biological implant" similar to the mechanical and direct implantation that is presently carried out in accessible tumour-bearing sites. It seems realistic to predict that future programmes of integrating immunology, nuclear medicine, radiation physics, medical oncology and radiotherapy may produce major achievements.

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