Acta Oncologica Vol. 32, Nos. 7/8, pp. 741–746, 1993

TARGETED IMMUNOTHERAPY

An update with special emphasis on ovarian cancer

ANTHONY MARAVEYAS and AGAMEMNON ANTHONY EPENETOS

An overview of antibody-guided immunotherapy for neoplasia is presented. The diversity of the antibody molecule is highlighted, through the many sophisticated strategies proposed and employed, to overcome a number of problems impeding successful targeting. An update of radioimmunotherapy of ovarian cancer is presented and the exciting concepts that are introduced to the field of targeted cancer therapy by molecular biology and genetic engineering are addressed.

Ovarian cancer causes clinical symptoms usually at an advanced stage, when treatment is not effective and prognosis is poor. Despite initial promise CA 125 has not proved a successful screening marker (1), and no means of identifying patients with early stages of disease are currently available. Three and a half thousand women in England and Wales and 12 500 in the USA die of the disease each year (2). It has currently evidenced that aggressive surgical debulking and platinum-based chemotherapy have improved response rates, but have failed to substantially increase survival (3). Moreover, it is well recognized that poor local control is the main cause of treatment failure, and strategies designed to deliver more treatment locally are the focus of intense clinical research. A number of agents have been used for intraperitoneal therapy, i.e. classical chemotherapeutic agents (4), cytokines (5), radiocolloids (6), immunoadjuvants (7) and monoclonal antibodies (8).

The monoclonal antibody, which embodies the promise of specific therapy, is by far the most diverse, not least for the numerous modification possibilities that the use of molecular biology technology promises. In this review we will outline the current status of antibody treatment in neoplasia and then focus on the advances in the field specifically concerning ovarian cancer, simultaneously highlighting some of our own experience in treating this malignancy.

Received 8 February 1993.
Accepted 22 April 1993.
From Imperial Cancer Research Fund, Oncology Group, Department of Clinical Oncology, Royal Postgraduate Medical School, Hammersmith Hospital, DuCane Rd., London, W12 0HS, England (both authors).
Correspondence to: Dr A.A. Epenetos (address as above).
Presented at the 3rd Scandinavian Symposium on Monoclonal Antibodies in Diagnosis and Therapy of Cancer, October 30–31, 1992, Helsinki, Finland.

The target

Epithelial neoplasms. Heterogeneity of antigen expression, poor perfusion of large tumour areas, exuberant stromal reaction, dense cellularity, and high interstitial pressure due to lack of lymphoid vessels are just a sample of the known problems posed to targeted immunotherapy by the anatomic and pathological properties of the solid tumour. It is unlikely that targeting of each and every cancer cell will be achieved. However, immunomodulation strategies, aimed at increasing antigen expression (9), manipulation of microvasculature through vasoactive substances (10), or using complex toxins (radioimmunotoxins) (11) with built-in toxin activity designed to kill the targeted cell while at the same time carrying a radionuclide that can irradiate antigen negative cells through 'crossfire' effects, may improve currently achievable results in targeted immunotherapy. Furthermore, loco-regional administration schemes may achieve higher antibody concentrations at solid tumour sites confined to cavities (12). Overcoming human antimouse antibody (HAMA) responses by the introduction of humanized/human antibodies (13) may allow for multiple treatments which may improve the prospects for treating bulky tumours. Using

the antibody as part of a multimodality treatment strategy early in the initial intervention, when it might be maximally effective in minimal residual disease and not as a last resort treatment merits consideration.

Haemopoietic malignancies. Some of the most promising responses have been obtained in malignancies derived form haemopoietic cells (14–16). Haemopoietic solid tumours have proved remarkably sensitive to low dose rate irradiation as achieved by radioimmuno-therapy and are relatively more sensitive to external beam radiotherapy (17). Although the reasons are not clear it is postulated that apoptosis (programmed cell death) is easily induced in lymphoid malignancies (18). This characteristic coupled to the fact that bone marrow ablation is an acceptable therapeutic option has led to much higher radiation and toxin doses being administered and a subsequent increase in the therapeutic efficacy (19).

Elements

The monoclonal antibody. The promise of specific cancer therapy, which the antibody left in its wake, still has a number of hurdles to overcome. Absolute tumour-specific antigens are rare and so cross-reactivity is a potential problem. Non-specific targeting of tissues rich in reticuloendothelial tissue (bone marrow, liver) is of concern. Penetration into solid tumours, due to adverse physiological or anatomical properties already mentioned, is poor (20). Not even issues of the impact of antibody affinity or avidity or antigen density on therapeutic potential find researchers in agreement (21, 22). A sensitive host immune system is a further impediment as, apart from the well-characterized response to HAMA, it can also recognize even relatively small chemical alterations to humanized molecules caused by linkers (23), thus limiting any therapeutic potential of repetitive treatment. Absolute uptake in human cancer is much less than that achieved in murine xenografts (24). The use of antibody fragments has been an option heavily investigated. They have a faster clearance from the blood stream and lead to better tumour-to-normal tissue ratios. However, absolute amounts in tumours are actually lower than those achieved by intact IgG, which in turn reduces therapeutic potential of 'one-off' treatment. although it is not yet clear which is the smallest antibody fragment to achieve the balance between blood clearance and absolute tumour uptake, $F(ab)'_2$ fragments seem to be good candidates (25). It is also conceivable that the best candidate for loco-regional immunotherapy may be the IgM molecule (25), as its slower clearance to the periphery may allow for longer resident time of the toxic agent in the cavity.

Toxic agents. Direct cellular toxicity may be mediated by the lone antibody. Mechanisms implicated are complement activation (26) and antibody-dependent cell cytotoxicity (ADCC) (27). Antibodies mediating apoptosis have also been developed (28), while antibodies against growth factor receptors may also adversely affect tumour growth (29). Nevertheless, investigators generally agree that for efficient immunotherapy the mAb has to either deliver a toxic agent to the tumour, or deliver a molecule that can locally activate/amplify a tumouricidal process.

Radionuclides. ^{131}I is the radionuclide initially used for radioimmunotherapy. Familiar and relatively straightforward chemistry, low cost and proven clinical efficacy in thyroid carcinoma are some of the considerations behind this choice. A number of shortcomings though, not least the enviromental hazards posed to the nursing staff, have led to the investigation of a number of other β-emitting nuclides. ^{32}P, another radionuclide with a successful clinical track record in the treatment of polycythemia rubra vera, is also receiving attention as a radioimmunotherapeutic agent. Kemptide a biologically active peptide which binds ^{32}P, has been conjugated to antibodies (30) and is on the verge of a clinical phase I study. Theoretical macro- and microdosimetry studies seem to indicate that the choice of emitters will have to be dictated by the therapeutic objectives and intent and not by matters of convenience, cost or availability (31). The theoretical implications are that short-range beta emitters, i.e. ^{131}I, will be more capable of sterilizing micrometastatic disease, while long-range β-emitters, i.e. ^{90}Y, may be capable of eradicating larger deposits only (32). Bulky disease seems still to be outside the capabilities of targeted radiotherapy, mainly due to shortcomings in the delivery system. It also seems that either using a number of radionuclides simultaneously or using a short range β-emitter in combination with external beam radiotherapy may prove a more efficacious way of 'sterilizing' over a wide range of cancer seedling volumes, designated as 'microscopic' residual disease (33). Promising nuclides exhibiting positive properties of ^{131}I while lacking the problematic high energy gamma ray seem to be ^{186}Re (34) and ^{153}Sm (35). However, obtaining a carrier-free product is a problem for the former (24) while a worrying bone seeking property, evident in preclinical experiments (36), may affect the therapeutic usefulness of the lattter. Questions of general availability and cost are other substantial drawbacks. Nonetheless simple labeling chemistry and promising physical properties coupled to low-energy imageable gamma rays, make these nuclides promising dual purpose candidates. Another area where improvements are needed is that of chelator technology. Inability to administer even the amounts of radionuclide calculated to treat minimal disease, due to inordinate in vivo chelator instability, has often proved the case, e.g. yttrium-90-diethylene triamine pentaacetic acid (DTPA)-antibody conjugates (37).

Antineoplastic drug and biological toxins. A diverse array of possible toxic agents can be chemically conjugated to antibodies. Most of the currently used chemotherapeutics have sometimes been coupled to an antibody (38).

Preclinical studies have shown that targeting of the antibody-drug construct is often as efficient as that of the lone antibody, as these drugs are often small molecules. However, in vivo and in vitro studies indicate that the drug activity of the construct is significantly reduced, bioavailability is subject to the paharmacokinetic shortcomings of the antibody, and few drug molecules per antibody can be delivered. This has led to the investigation of more promising agents, such as biological toxins that can kill a cell per molecule. Problems of even small or low-affinity carrier cross-reactivity will become more apparent due to enhanced toxicity.

Two-step strategies

The effort to achieve either absolute or relative amplification of the tumouricidal effect, through the increase in the generation or acquisition of reactive molecules at the tumour site or a reduction of the toxic molecules available to the periphery, has led to a number of strategies, having in common the approach of splitting treatment into non-toxic carrrier phase and a toxic treatment phase.

Bifunctional/bispecific antibodies. Bifunctional antibodies, in which each antigen-binding site recognizes a different antigen, may be used for targeted cancer therapy if one binding site recognizes a tumour antigen and the other a cytotoxic agent (39). It is also possible for the other binding site to mediate T-cell recognition and target cell lysis (40), while the local release of cytokines may induce bystander cell growth inhibition (41).

Streptavidin-biotin. A two-step approach to guided radioimmunotherapy is illustrated by the avidin biotin system (42); briefly, a streptavidin conjugated mAb (modified antibody) is infused, allowed to either localize to tumour or be cleared from the body (first step). This prolonged but non-toxic phase is followed by the administration of a biotin-conjugated radionuleotide (second step). The extremely high affinity and specificity of biotin for streptavidin ($K_d = 10^{-15}$ M) and its fast clearance kinetics due to its small molecular weight lead to enhanced tumour-to-normal tissue ratios. Currently a three-step approach has received some investigation (43), where a biotinylated antibody is infused as a first step followed by an infusion of a 10-fold excess of streptavidin (second step). The rationale is to 'chase' the antibody from the circulation as the liver clears antibody-streptavidin complexes very quickly and avidinate the antibody that has bound to the tumour. This achieves the objective of being able to give the labelled biotin (third step) 24–48 h after the antibody administration, when tumour uptake of the antibody is maximal, simultaneously reaping the benefit of the fast clearance of this small molecule. These modifications hold promise for improvements in radioimmunoscintigraphy but it is doubtful whether absolute amounts in the tumour will be high enough for successful radioimmunotherapy. One must also bear in mind the fact that streptavidin is a highly immunogenic molecule and so multiple applications may be limited.

Pro-drug activation strategies (ADEPT). Briefly an antibody-enzyme conjugate (first step) is injected into the body, allowed to target or clear from the circulation. Following this, the substrate is administered (second step), which is a non-toxic prodrug, but converted at sites of enzyme activity into a toxic compound. Although the same pharmacokinetic and biodistribution shortcomings, as described for the lone antibody, persist and problems of steric hindrance or reduced antibody immunoreactivity may be introduced, the possibility of achieving an enzyme-mediated amplification effect holds promise of delivering a large number of active molecules to the tumour site (44).

Practical applications

Experimental immunotherapy. The xenograft model in nude mice has been widely used for experimental immunotherapy. It allows for extensive study of some of the necessary prerequisites for successful immunotherapy. Some principles have been laid: The need for antibody internalization for effective action of toxins (45) and Auger emitters (46) and the effect of affinity on mediated toxicity (47) are but a few examples. Promising imaging and treatment results have been consistently achieved (48). A number of models exist for systemic and regional treatment. However, the extrapolation of these successful results to the human model have not been so gratifying. Many toxicity profiles have not been acurately predicted, nor have dosimetric and radiobiological assumptions been realized (49). Nevertheless, these promising experimental results cannot be discarded and they give a prevue of what optimization of this form of treatment may hold for the cancer patient in the future.

Clinical immunotherapy. In the midst of all these issues antibody immunotherapy has made tentative steps forward in clinical practice. Immunotoxins have been used in haemopoietic malignancies with encouraging results (50). Radioimmunotherapy, especially when used at myeloablative doses with bone marrow rescue, has also produced encouraging results in lymphoproliferative malignancies (19). Solid tumors have also been treated with a number of immunotherapeutic agents. Patients with melanoma (51), colorectal carcinoma (52), and a variety of other solid carcinomas have been treated with immunotoxins in phase I trials without any clinical antitumour responses being evident. Ongoing trials of radioimmunotherapy of a number of solid tumours have produced a number of palliations of effusions (12), complete and partial responses (53, 54), more commonly in minimal disease. However, the fact that most of these patients were heavily pretreated individuals with widespread bulky disease encourages further research into the refinement of these strategies.

Ovarian cancer

Most of the previous exposition concerned antibody treatment of cancer in general, the problems that exist and the strategies that have been devised to overcome some of these problems, also pertain directly to ovarian cancer. This malignancy is a model well suited for the investigation of regional immunotherapy. Our group originally used ^{131}I coupled to a variety of antibodies against ovarian cancer. Some encouraging results in small-volume disease were obtained (8). Later yttrium-90 became the agent of choice and the chelating technology a focus for improvement. DTPA, the original chelating agent, was not stable enough in vivo (37), while the chelating macrocycle DOTA, which had very promising in vitro properties, had significant immunogenicity (55) and clinical side-effects in 50% of patients treated (56). A phase I toxicity study, assessing (S)-4-[2,3-bis[bis (carboxymethyl) amino propyl] isothyocyanate of DTPA (CITC.DTPA), another chelating agent, has recently been completed. It emerged that this molecule, despite not being a macrocycle, is also serologically immunogenic (manuscript in preparation, Kosmas et al.) in a significant proportion of patients. Clinically important but self-limiting side-effects were observed in 5 of the 19 patients treated so far. At least twice the amount of radioactivity can be conjugated with this agent, as compared with DTPA and a relation between radiation dose per square meter of body surface area and bone marrow toxicity was observed (64). Retrospective analysis of all the patients treated with this regimen, irrespective of chelating agent, produced therapeutically interesting results in patients with negative second-look laparoscopy (65), and a randomized phase III study in patients in clinical remission receiving this treatment is currently being initiated. The availability of a humanized antibody may see the initiation of a phase I trial soon. A number of other groups are also evaluating the intraperitoneal route for treatment of ovarian carcinoma, reporting results similar to those originally reported by our group (57, 58). It is often difficult to reconcile some of the results obtained with theoretically inadequate radiation dose administered, and the consideration that the anitbody may be exerting an effect through an anti-idiotype immunizing mechanism is of considerable interest (59).

Combining intravenous and intraperitoneal treatment is a strategy worth pursuing (60) as uptake in intraperitoneal implants may be increased if the antibody is given intraperitoneally but retroperitoneal disease may not be targeted satisfactorily (61). The approach of combining both routes to administer a radioimmunotoxin is being evaluated in preclinical models (11). The clinical experience from two pilot studies assessing rhenium-186 labelled monoclonal antibodies included 2 patients with ovarian cancer (62). Treatment was administered intravenously and maximum tolerated activity was 3300–4600 MBq/m^2 of body surface area depending on the intensity of prior treatment schedules. Bone marrow toxictiy was the dose limiting factor. More radioactivity could be administered using the F(ab)$'_2$ fragment of the antibody. Marrow dose limiting toxicity was not reached even when activities of 7400 MBq/m^2 were administered. However, an increased incidence of perturbation of liver function tests was noted.

Results on a phase I immunotoxin therapy trial (63), evaluating the pseudomonas exotoxin immunoconjugate OVB3-PE administered intraperitoneally in 23 patients with ovarian carcinoma, concluded that high doses of immunotoxin can be administered via this route. Bone marrow toxicity was not reached but neurotoxicity proved to be the dose-limiting factor postulated to be due to cross-reactivity of the antibody carrier to granule cells in the cerebellum. This study highlighted the anticipated result of toxicity becoming manifest even in a low affinity cross-reactivity setting, due to the enhanced toxicity of the toxin. Pseudomonas exotoxin was highly immunogenic, all patients developing antibodies against domain II of the pseudomonas exotoxin molecule and HAMA within 14 days of treatment; no responses were observed.

New frontiers

Improvements are being sought in all aspects of cancer ranging from prevention to prognosis and treatment. Despite non-realization of many of the high expectations which the investigators held for immunotherapy when the monoclonal antibody was discovered, we believe that targeted cancer therapy is still the way forward. Molecular biology technology holds great promise for modification of the anitbody molecule. Engineered immunotoxins and bispecific antibodies will solve the problem of non-standardized biochemical preparations. Humanized antibodies will be easier to manufacture. A wealth of composite designer molecules, based on the antibody specificity but having built-in multiple effector molecules, can be envisaged. we are currently being catapulted into the gene therapy era, and it is to be hoped that the lessons learnt about how the antibody functions, and to what extent it could be modified and still have a functional unit, will hold us in good stead even in targeted gene therapy.

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