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Commentary

Targeted immunotherapy of cancer: development of antibody-induced cellular immunity

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Abstract

Although immunotherapy of cancer encompasses a large variety of distinct protocols, virtually all therapeutic strategies require the enabling/training of the immune system to distinguish tumour tissue from healthy tissue. In the case of antibody-based therapies, specificity obviously arises from the selectivity of the antibodies for tumour antigens, and tumour cell death derives from either direct cytotoxicity of the antibody or antibody-dependent cellular cytotoxicity. However, even when both of the above killing mechanisms are simultaneously active, we suggest that antibody-based immunotherapies may fall far short of their full potential. In this editorial, we first summarize the mechanisms by which current antibody-based therapies mediate cancer cell removal, and then propose two strategies by which this class of immunotherapies might be further improved. These suggested improvements involve the decoration of tumour cell surfaces with foreign haptens against which an endogenous humoral immune response can be mounted and the recruitment of the cellular arm of the immune system in an antibody-dependent process.

Introduction

Believing that complete elimination of malignant cells must ultimately require participation of the patient's own immune system, a variety of immunotherapeutic strategies have emerged that focus on recruiting immune components to the site of tumour growth. These strategies include administration of tumour vaccines (Hsueh 2001; Mitchell 2002), tumour-specific T cells (Thomas & June 2001; Kessels et al 2002), tumour antigen-primed dendritic cells (Brossart et al 2001), heat-shock protein-based vaccines (Manjili et al 2002), cytokine-expressing gene therapy vectors (Morse 2000) or DNA vaccines (Nawrocki et al 2001; Nishioka et al 2002), immunotoxins (Kreitman et al 2001), bispecific antibodies that cross-link cancer cells to $CD3^+$ lymphocytes and Fc receptor-positive immune cells (Ruf & Lindhofer 2001; Withoff et al 2001), and unmodified tumour-specific monoclonal antibodies (Linenberger et al 2002; Sondel & Hank 2001). While most of these strategies are still undergoing clinical and preclinical development, treatment with tumour-specific monoclonal antibodies has recently earned the distinction of becoming the most common treatment for nonresectable tumours next to chemotherapy and radiotherapy. Due to this newfound prominence and the associated interest in this technology, we have decided to focus our commentary on the possible mechanisms of action of antibody-based therapies and potential strategies for the further improvement of such therapies.

Modes of action of tumour-specific monoclonal antibodies

Treatment of cancer patients with monoclonal antibodies (mAbs) to tumour-specific cell surface antigens is thought to promote tumour regression by several distinct mechanisms. Firstly, unmodified mAbs may directly induce tumour cytotoxicity by blocking the functions of essential cell-surface signalling receptors, as has been found for trastuzumab (Herceptin) that targets the human epidermal growth factor receptor-2 (HER-2) (Baselga et al 2001). Alternatively, mAb binding to tumour cell surface receptors can trigger apoptosis by activating programmed cell death pathways linked to the targeted receptors (Maloney et al 2002). Thirdly, through their interactions with Fc receptor-expressing immune cells (particularly natural killer cells, monocytes, macrophages, and neutrophils), mAbs may trigger antibody dependent cellular cytotoxicity

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(ADCC), where engagement of the Fc receptor is seen to activate killing of the antibody-coated tumour cells (Sondel & Hank 2001). To a lesser degree, mAbs may also induce tumour cytolysis through complement fixation and the subsequent emergence of complement dependent cytotoxicity (CDC), which should lead to recruitment of phagocytic cells and their involvement in tumour destruction (Maloney et al 2002). Moreover, certain mAbs are useful as anti-idiotype vaccines (Bhattacharya-Chatterjee et al 2001), since they can mimic tumour-associated antigens and thereby enhance the ability of the host's immune system to identify the tumour as foreign. Finally, mAbs have been observed to enhance direct phagocytosis of tumour cells, a process through which subsequent long-term cellular immunity involving cytotoxic T cells (CTL) has been proposed to develop (Falo et al 1995; Selenko et al 2001). While not all mAbs clearly mediate their tumour responses through all of the above mechanisms, most successful mAbs probably act by more than one mode of action. As discussed below, we suggest that the most potent mAb-based therapies will elicit participation of the cellular immune system in locating, attacking, and eliminating malignant cells.

Mechanisms of antibody induction of tumour-specific cellular immunity

As alluded to above, there is mounting evidence that tumour-reactive antibodies can enhance a tumour's susceptibility to cytotoxic T cell-mediated cytolysis (zum Buschenfelde et al 2002). Although their relative contributions remain unresolved, two specific pathways are thought to account for most of this cross stimulation. First, it has been suggested that mAbs (e.g. Herceptin) might promote the endocytosis and subsequent degradation of their cognate antigens (such as HER-2). Peptides derived from the digested tumour antigens might then be processed and presented by available MHC class I molecules, leading to expansion of tumour-specific CTL clones and the consequent tumour lysis by the cytotoxic T cells (zum Buschenfelde et al 2002). Unfortunately, while such an antigen-processing pathway is clearly possible, tumour cells are generally not efficient antigen-presenting cells, often because they express reduced levels of MHC molecules or lower numbers of the co-stimulatory polypeptides required for T cell activation (Schultze et al 1996).

Alternatively, recent reports indicate that antitumour antibodies may facilitate uptake and presentation of tumour antigens by professional antigen-presenting cells (APCs) in a process referred to as "cross-priming" or "cross-presentation" (Amigorena 2002). In this process, APCs such as dendritic cells (DCs), macrophages, and monocytes migrate through the tumour tissue until they encounter antibody-coated tumour targets. Fcγ receptors (CD64, CD16/CD32) expressed on these APCs then mediate uptake of the antibody-coated tumour cells via receptor-mediated endocytosis (for soluble immune complexes) or phagocytosis (for antibody-opsonized particles and cells) (Booth et al 2002). Internalization and processing of the antibody-coated tumour cells can then lead to cross-presentation of any tumour-associated antigens and the subsequent activation of tumour-specific cytotoxic T cells (Schuurhuis et al 2002). Thus, the decoration of tumour cells with tumour-specific antibodies and the subsequent phagocytosis of the marked cells by Fcγ receptor-expressing APCs may constitute an efficient method for recruiting direct participation of the cellular arm of the patient's immune system.

Evidence for the occurrence of cross-priming by tumour-specific antibodies

In animal tumour models, mAb-mediated tumour regression and protective immunity have been shown to involve Fc receptors and the participation of tumour-specific cellular effectors of the immune system (Vasovic et al 1997; Clynes et al 1998; Dyall et al 1999). Although direct evidence for tumour-specific CTLs has not been established in human cancer patients treated with antitumour mAbs, in-vitro experiments with isolated cells strongly suggest that such cross-priming can occur. Thus, in studies with Rituximab, an FDA-approved chimeric mouse/human mAb that targets the CD20 antigen on non-Hodgkin's B-cell lymphoma, Selenko et al (2001) found that the mAb-induced apoptosis of the CD20-positive lymphoma cells specifically promoted phagocytosis by dendritic cells (DCs), and that co-culture of such tumour-loaded DCs with autologous T cells induced an efficient CTL response against lymphoma-associated antigens. To characterize this process further, Dhodapkar et al (2002) loaded DCs with apoptotic, necrotic or antibody-coated tumour cells and compared their abilities to promote expansion of antigen-specific CTLs. They showed that dendritic cells that phagocytosed antibody-coated tumour cells were more efficient in cross-priming CTLs than simple DCs that had phagocytosed apoptotic or necrotic tumour cells. The cross-presentation of tumour antigens was shown to be mediated by interactions with Fcγ receptors and to be further enhanced when the tumour cells were induced by an antibody to undergo apoptosis (Dhodapkar et al 2002). Finally, to partially characterize the mechanism of antibody-mediated cross-priming of CTL, Schuurhuis et al (2002) studied the maturation and cross-priming capacity of DCs during exposure to antigen–antibody complexes. In-vitro results indicated that immune complexes could both activate DCs by inducing maturation as well as enhance their abilities to present exogenous antigens on MHC class I molecules. In-vivo, the immune complex-treated DCs were found to efficiently prime specific $CD8^+$ CTL responses without any help from $CD4^+$ T helper (Th) cells, an indication of a Th-independent CTL activation mechanism (Schuurhuis et al 2000).

Not surprisingly, macrophages (and monocytes) are not considered as important in promoting tumour antigen cross-priming as DCs. In fact, the targeting of antigens via Fcγ receptors to activated macrophages may even reverse their innate Th1-like bias and stimulate production of

antigen-specific T cells that produce Th2-like cytokines (Anderson & Mosser 2002). Nevertheless, macrophages and monocytes still represent important effector cells in many antibody-mediated therapies, since they participate in both ADCC and phagocytosis of antibody-marked tumour cells. Macrophages may also enhance recognition and attack of tumour cells by CTLs, since the targeting of antigens to Fcγ receptors on such myeloid cells has been shown to enhance killing of the opsonized cells by antigen-specific CTL (Wallace et al 2001). Finally, Kroesen et al (2002) reported that tumour-specific antibodies were capable of activating myeloid cells via interactions with their high affinity Fcγ receptors, which in turn could upregulate adhesion molecules on adjacent endothelial cells, at least in-vitro. Assuming that a similar process occurs in-vivo, formation of mAb-containing immune complexes at the tumour site should promote local inflammation, immune cell migration, and activation of other APCs, all of which can help promote development of a cellular immunity against the cancer.

Strategies for improving antibody-based cancer therapies

Despite the tremendous success of antibody-based therapies in man to date, we suggest that two modifications should lead to further improvements in therapy. First, as noted above, involvement of the cellular arm of the immune system as an equal partner with the mAb-mediated therapy would be highly desirable. In general, however, specific measures to maximize cellular involvement have not been explored. Based on the mechanisms of cross-priming summarized above, we suggest that immune stimulants that enhance MHC expression, co-receptor expression, antigen presentation or CTL activation should be included to promote CTL involvement. Obvious candidates for this priming function might be Th1 cytokines (e.g. interleukin-2, interleukin-12, interferon-α), immunostimulatory oligonucleotides (e.g. CpG-containing oligos), or Th1-biasing adjuvants (e.g. saponin derivatives such as QS-21, GPI-0100, etc.). With the mAb promoting phagocytosis/endocytosis of the tumour antigen and the immune stimulant assuring efficient antigen processing and presentation, the potential for CTL recruitment should be greatly enhanced.

A second improvement might derive from allowing the patient to produce and maintain his or her own tumour-specific antibody titre throughout the course of therapy. While vaccination against endogenous tumour antigens has long been explored as a mechanism to elicit anti-tumour immunity, success with such vaccines has been limited by the tolerance that develops to the endogenous tumour antigens during the early stages of tumour growth. While much effort is justifiably being devoted to strategies for breaking this tolerance, an alternative solution might be to introduce onto the tumour cell surface a neo-antigen that the body has either not seen before or has already developed a strong immunity against. Conceivably, one could decorate a tumour cell surface with a small molecular weight hapten like the active ingredient in poison ivy, urushiol (Kalish & Wood 1997). However, given the likely aversion of many former poison ivy sufferers to an injection of urushiol, we have elected instead to employ the intrinsically benign, but highly antigenic molecule, fluorescein. In this effort, Lu & Low (2002) have linked fluorescein to the vitamin folic acid, which in turn binds with high affinity ($K_d \sim 10^{-10}$ M) and in large numbers to tumour cells that over-express a receptor for folic acid (cancers that over-express the folate receptor include malignancies of the ovary, kidney, brain, lung and breast (Ross et al 1994; Weitman et al 1994; Toffoli et al 1997)). Thus, within minutes of intravenous injection of the folate–fluorescein conjugate, tumour cells become densely decorated with fluorescein, while folate receptor-negative normal tissues remain hapten free. In animals pre-immunized against fluorescein, tumour cells then become rapidly coated with anti-hapten antibodies, leading to antibody-mediated tumour cell regression. No difficulties with immune tolerance have been detected because even immune compromised animals mount and maintain a humoral immune response against the strongly foreign hapten, fluorescein. Further, antibody titres are seen to remain high for months, obviating the need to continually inject costly mAbs. Finally, this two-stage antibody targeting strategy has been adapted further to enable simultaneous mobilization of the cellular arm of the immune system (Lu & Low 2002). Thus, according to the proposal above, it has been observed that co-administration of immunostimulatory cytokines, such as those that activate immune effector cells and/or enhance tumour antigen presentation (e.g. interleukin-2 and interferon-α) induce strong CTL involvement with an accompanying long-term memory, preventing tumour cell colonization following subsequent rechallenge with the same cancer (Lu & Low, 2002). These results suggest that the hapten-mediated killing/removal process has triggered local APCs to internalize and present tumour cell components to T cells, which in turn somehow recognize a unique tumour antigen among the various components presented.

Conclusions

A major goal of cancer immunotherapy is to induce a tumour-directed immune response that is capable of eliminating existing tumour masses and establishing long-lasting protective immunity against recurrence of the same cancer. For most applications involving antitumour antibodies, ADCC is still considered the predominant effector mechanism, probably because its potency can be easily demonstrated in-vitro. Mechanistically, however, antibody binding to tumour cells can also trigger cross-presentation of tumour antigens to local Fcγ receptor-positive APCs, which can then reprogram the cellular arm of the immune system to recognize and destroy residual tumour cells. The cellular arm of the immune system is often not prepared to respond in these situations and so the immune stimulants that enhance antigen processing/presentation can aid in recruitment of a cellular immunity. Since co-participation of both humoral and cellular arms of the immune system

should lead to more rapid and thorough elimination of tumour tissue than either arm alone, modifications to recruit cellular involvement should improve response rates. Further improvements in response rates can be anticipated whenever anti-tumour antibody titres can be maintained by the patient's own immune system at a high level for extended periods (as will likely be the case for antibodies raised against foreign haptens).

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