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Fighting Cancer with Monoclonals and Autoimmune Diseases with T-cell Epitopes

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Antibodies to HER-2/Neu proto oncogene can either stimulate or inhibit tumor growth. The inhibitory antibodies convert a breast cancer cell into a normal cell producing casein and lipids (milk ingredients). A synthetic amino acid copolymer, denoted COP-1, can suppress the onset of allergic encephalomyelitis in animals, revert the disease in monkeys, and arrest or even revert the exacerbating-remitting form of multiple sclerosis in humans. Thus COP-1 serves as an immunomodulatory vaccine against an autoimmune disease. A similar approach is being used now to myasthenia gravis.

Key Words: Breast cancer, New proto-oncogene, Multiple sclerosis, Myasthenia gravis, Vaccination against, Autoimmune diseases

Introduction

Immunology has been very successful in fighting infectious diseases through vaccination, but it has also an enormous potential, largely still untapped, in the immunotherapy of cancer—through increasing immunity—on the one hand, and of immunotherapy of autoimmune diseases—through diminishing specific immune responses on the other. Examples in both directions will be given below.

Anti-Neu Antibodies can either Stimulate or Inhibit Tumor Growth

The HER-2/Neu proto-oncogene (also called ERBB-2) encodes a tyrosine kinase receptor for a polypeptide growth-regulatory molecule. Amplification and overexpression of the gene have been frequently observed in human adenocarcinomas and correlated with poor prognosis. To explore the potential of antibody therapy directed at the HER-2/Neu receptor, we have raised a panel of murine monoclonal antibodies specific to the extracellular portion of the human protein, and tested their effect on the tumorigenic growth of HER-2/Neu transfected fibroblasts in athymic mice (Stancovski et al. 1991, Bacus et al. 1992).

Surprisingly, opposing *in vivo* effects were observed: although some antibodies almost completely inhibited the growth in athymic mice of transfected murine fibroblasts that overexpress ERBB-2, other antibodies either accelerated tumor growth or resulted in intermediate responses. When tested on cultured human breast carcinoma cells or ERBB-2 transfectants, the tumor-stimulatory antibody was found to induce significant elevation of tyrosine phosphorylation of the ERBB-2 protein. In contrast, only partial correlation was observed between the

capacity to restrict tumor growth and the effects of the antibodies on receptor degradation and cellular proliferation *in vitro*. This suggests that the antitumor antibodies affect both receptor function and host-tumor interactions. The different effects are mediated by distinct epitopes on the receptor molecule (Stancovski et al. 1991).

The above results may point to a *specific* immunomodulation of tumor growth by means of monoclonal antibodies, and they may help establish experimental criteria for the selection of specific antibodies for use either alone or in conjunction with other molecules as pharmacological antitumor agents.

Differentiation of Breast Cancer Cells into Normal Milk-producing Breast Cells

The opposing effects on growth described above, were induced by the various monoclonal antibodies, whether they were injected intraperitoneally (Stancovski et al. 1991) or intravenously (Bacus et al. 1992). To understand the cellular mechanisms underlying antibody-induced tumor inhibition, we tested the effect of the monoclonal antibodies on various cultured human breast cancer cells (Bacus et al. 1992). Our analysis revealed that the tumor-inhibitory antibodies specifically induced phenotypic cellular differentiation that included growth arrest at late S or early G_2 phase of the cell cycle, markedly altered cytoplasm and nuclear morphology, synthesis and secretion of milk components (casein and lipids), and translocation of the HER-2/Neu protein to cytoplasmic and perinuclear sites. The extent of cellular differentiation by various antibodies could be correlated with their tumor-inhibitory potential, whereas a tumor-stimulatory monoclonal antibody or control immunoglobulin were completely inactive with respect to cellular differentiation.

It is worthwhile to review the landmarks of the differentiated phenotype that was induced by the antibodies in breast tumor cells. This state appears to be analogous to mature breast cells which secrete milk components such as casein and lipids. In addition to their flat morphology and large nuclei, the antibody-treated cells were also growth arrested, as was reflected by their DNA ploidy and cell proliferation. The mechanism by which an antibody to a receptor tyrosine kinase promotes cellular differentiation is unknown. It has been previously observed that activation of certain receptors, including HER-2/Neu, confers competence of mouse mammary cells to differentiation by lactogenic hormones (Hynes et al. 1990, Taverna et al. 1991). Moreover, transgenic mice in which c-myc expression was targeted to the mammary glands constitutively transcribed casein genes (Schoenenberger et al. 1988). Thus, tumor growth and cellular differentiation, at least as reflected by milk production, are not mutually exclusive in the mammary gland. One possibility, which has not been examined by us, is that the antibodies induce differentiation only in conjunction with serum factor(s). An alternative possibility is that the antibodies interfere with the constitutive or ligand-induced mechanism of signal transduction by HER-2/Neu. Accordingly, this inhibition directs the cell into the differentiation pathway. Mechanistically, inhibition of signal transduction could be mediated by dissociation of homodimers of the HER-2/Neu receptor and a consequent inhibition of its constitutive- or ligand-induced elevated tyrosine kinase activity (Lonardo et al. 1990, Peles et al. 1991). Our monoclonal antibodies accelerated endocytosis and degradation of the receptor protein (Stancovski et al. 1991), and more important, only the tumor-inhibitory MAbs induced redistribution of the receptor. The significance of the observed translocation from the membrane to the nucleus is also not understood. Apparently it eventually leads to

receptor degradation, but this may be slightly delayed as to allow the existence of undergraded receptors within the cytoplasm. We speculated that the translocation process actively or passively permits the cell to enter the differentiation pathway. The molecular nature of this process, however, remains largely unclear, except for the fact that activation of protein kinase C may independently initiate it (Bacus et al. 1990), in analogy with the EGF receptor (Lin et al. 1986).

Taken together, our *in vivo* and *in vitro* studies correlated the tumor inhibitory potential of monoclonal antibodies to HER-2/Neu with their capacity to induce cellular differentiation *in vitro*, and raise the attractive possibility that the intrinsic capacity of certain monoclonal antibodies to HER-2/Neu to induce such terminal differentiation of human adenocarcinoma cells is mechanistically correlated with their tumor-inhibitory action (Bacus et al. 1992). This observation may be clinically important as it offers a strategy for immunotherapy of the many types of cancers that involve overexpression and amplification of the HER-2/Neu oncogene, and even tumors that express relatively low levels of the protein.

Immunomodulatory Vaccines against Autoimmune Diseases

Besides vaccines against infectious diseases, we must consider now another type of vaccination, namely, vaccination against autoimmune diseases (Sela & Arnon 1992). In our own work over more than two decades, we have demonstrated that a synthetic amino acid copolymer, denoted COP-1 (Teitelbaum et al. 1973), is capable of suppressing the onset of allergic encephalomyelitis in experimental animals, reverting the disease in simians (Sela et al. 1992) and either arresting or reverting the progress of the exacerbating-remitting form of multiple sclerosis in humans (Bornstein et al. 1987). While COP-1 is, therefore, a polymeric drug, it is at the same time an immunomodulatory vaccine.

In recent years, a more general approach has been developed by Cohen and his colleagues (Cohen 1986, 1989). Vaccination of autoimmune patients with subpathogenic doses of T cells specific for the autoantigen, may ameliorate autoimmune reactions, possibly through the generation of 'anti-clonotypic' T cells that inhibit the patient's autoreactive T cells. T-cell vaccination may be compared to the use of attenuated or avirulent microbes specifically for immunization against the disease caused by that microbe in its virulent form; however, in this case the vaccine is a population of T cells, and the pathogens to be controlled are endogenous clones of autoimmune lymphocytes. In T cells vaccination, the antigens are specific T cells and the responders are T cells or, more specifically, autoimmune T cells recognizing autoimmune T cells. The most recent and interesting example of this approach is the vaccination against autoimmune mouse diabetes with a T-cell epitope of the human 65 kDa heat shock protein (Elias et al. 1991).

Treatment of Multiple Sclerosis with COP-1

A synthetic copolymer of L-alanine, L-glutamic acid, L-lysine and L-tyrosine in a residue molar ratio of 6.0 : 1.9 : 4.7 : 1.0, denoted COP-1, has been shown by us to suppress experimental allergic encephalomyelitis in guinea pigs, rabbits, mice, rhesus monkeys and baboons (Sela et al. 1990), and to help patients with the exacerbating-remitting form of multiple sclerosis (Bornstein et al. 1987).

We observed already almost 20 years ago a slight cross-reaction between rabbit anti-COP 1 antibodies and bovine myelin basic protein (MBP) (Webb et al. 1973). At the cellular level, a marked cross-reaction was observed both *in vivo* in the delayed

hypersensitivity skin-test, and *in vitro* by measuring lymphocyte transformation. This cross-reactivity has been recently confirmed and investigated in detail, making use of monoclonal antibodies to MBP and to COP-1 (Teitelbaum et al. 1991). About a third of anti-rat MBP monoclonal antibodies and most of anti-mouse monoclonal antibodies cross-reacted with COP-1. In addition, several anti-COP 1 monoclonal antibodies cross-reacted with MBP. Moreover, some anti-MBP and anti-COP 1 monoclonals reacted in a heteroclitic manner, and favoured the cross-reactive antigen over the immunogen.

The suppressive activity of COP-1 correlates well with immunological manifestations. Thus an analog of COP-1 built exclusively of D-amino acids does not cross-react at all with MBP, and indeed exhibits no suppressive activity. Moreover, COP-1 does not suppress unrelated antibody formation or cellular responses, nor does it affect other autoimmune diseases.

After some preliminary studies with patients, a clinical double blind trial has been carried out (Bornstein et al. 1987). The participants had to have had at least three attacks in the previous two years. The 23 patients on placebo had during the trial period 64 attacks, whereas the 25 patients on COP-1 had only 16 attacks. The 13 less advanced patients had only 4 attacks (instead of the 39 expected). Thus, we have here a macromolecular drug candidate for multiple sclerosis, a drug prepared by polymeric techniques. A phase 3 double-blind trial with COP-1 is taking place now in 11 university, hospitals all around the United States. An open trial is taking place in four centers in Israel.

Possible Mechanisms of Action of the Polymeric Drug COP-1 in Multiple Sclerosis

Of course; it is of great interest to learn more about the mechanism of action of COP-1 in experimental allergic encephalomyelitis. One possibility is the induction of suppressor cells specific to MBP by COP-1 (Lando et al. 1981). More recently we have shown an additional immunological mechanism. Using MBP specific mouse T-cell lines and clones with various H-2 restrictions and antigen specificities, we have shown that, specifically, COP-1 could competitively inhibit T-cell responses to MBP (Teitelbaum et al. 1988). The effect of COP-1 on both the proliferative response and IL-2 secretion induced by MBP was followed in 8 T-cell lines and clones. In seven of them COP-1 specifically inhibited the response to MBP, and in one, COP-1 was able to induce proliferation. Inhibition of the response was shown to be specific to COP-1, and only T-cell line responses to MBP were affected by COP-1. These results suggest that COP-1 or COP-1-derived peptides can bind to the relevant MHC molecules and competitively inhibit the binding of MBP. Consequently, activation of MBP effector cells is blocked, while T-cells which cross-react with COP-1, e.g. suppressor cells, are activated. Thus, COP-1 may be effective in suppression of EAE not only because of selective stimulation of suppressor T-cells, but also by specific inhibition of MBP-specific effector T-cells.

The above findings were recently extended to the human MHC (Teitelbaum et al. 1992). COP-1 competitively inhibited the proliferative responses and interleukin 2 secretion of six BP specific T-cell lines and 13 clones of several DR restrictions and epitope specificities. Conversely, BP inhibited —albeit to a lesser extent—the response of all the COP-1 specific T cell lines and clones, irrespective of their DR restrictions. Another random copolymer of tyrosine, glutamic acid, and alanine, had no effect on these lines. Neither COP-1 nor MBP inhibited the response of PPD specific lines and clones. COP-1 and BP exerted their cross-

inhibitory effects only in the presence of antigen presenting cells. These results suggest that COP-1 can compete with BP for the binding to human major histocompatibility complex molecules. In view of recent studies implicating BP reactivity in multiple sclerosis, these findings suggest a possible mechanism for the beneficial effect of COP-1 in this disease. COP-1 and MBP exerted their cross-inhibitory effects only in the presence of antigen presenting cells, while incubation in their absence resulted in unresponsiveness to the homologous antigen. Thus, COP-1 competes with MBP for the binding to the MHC, but not to the T-cell receptor. It thus seems that the polymeric candidate drug against multiple sclerosis is essentially an immunomodulatory vaccine.

Possible Immunotherapy with Peptides Related to Myasthenogenic T-Cell Epitopes from Acetylcholine Receptor

We shall now have more and more cases where immunomodulatory vaccines will be used against autoimmune diseases. In our own laboratory we are working on myasthenia gravis (Katz-Levy et al. 1993). Two synthetic peptides, pp.195-212 and pp.259-271, representing sequences of the human acetylcholine receptor α-subunit, stimulate preferentially T-cells of patients with myasthenia gravis and are immunodominant T-cell epitopes in SJL and BALB/c mice, respectively. We designed and synthesized analogs of these peptides that contain single amino acid substitutions. An analog of peptide pp.195-212 (in which Met was replaced by Ala), was capable of inhibiting up to 100% of the proliferative responses of a T-cell line specific to peptide pp.195-212, originating from the high responder strain SJL. Similarly, an analog of peptide pp.259-271 (in which Glu was replaced by Lys), was capable of inhibiting up to 93% of the proliferative responses of the peptide specific T-cell line originating from high responder BALB/c mice. To test the *in vivo* inhibitory activity of the analogs, mice were primed with the myasthenogenic peptides in complete Freund's adjuvant concomitant with administration of the analogs in aqueous solution. Administration of the analogs led to decreased proliferative responses of up to 70% by peptide pp.199-212 primed lymph node cells, and up to 85% by peptide pp.259-271 primed lymph node cells. Similar results were obtained, whether the analogs were administered i.v. or i.p. Thus, these analogs are good candidates for specific immunomodulatory therapy for patients with myasthenia gravis.

Concluding Remarks

The examples I have brought forward here illustrate clearly that immunology already for decades has no longer been a science devoted exclusively to immunity against infectious diseases. Whether by increase in non-specific immunity, with various biological response modifiers, or whether by efforts to use specific antibodies, as described here, the role of immunity in fighting cancer is becoming apparent, even though it is still too early to assign it already a central role in combating this dreadful disease. There is no doubt, in my mind, nevertheless, that immunotherapy will in the near future be as important as radiotherapy and chemotherapy in our armamentarium against various types of cancer.

On the other hand, in the case of autoimmune diseases, the immunomodulatory vaccine approach has been shown already to be successful in the case of the treatment of the exacerbating-remitting type of multiple sclerosis with COP-1, and—most probably—as the result of intensive research in many laboratories, including our own, this will be extended soon to additional autoimmune diseases.

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