Immunoprevention of Cancer: Is the Time Ripe?¹

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Abstract

Immunotherapy applied to patients with established tumors rarely leads to an objective response, whereas patients apparently free from disease after conventional treatment and at risk of recurrence are beginning to receive vaccination. New classes of patients or not-yet patients are those with a high genetic or environmental risk of developing cancer. They may draw benefit from a “soft” treatment such as vaccination. This overview discusses the prospects of immune stimulation as a means of cancer prevention by inducing various forms of nonspecific or even specific immunity. Atainment of this goal provides the rationale and motivation for embarking on such a new and potentially rewarding enterprise.

Immunotherapy is emerging as an effective way to cure cancer (1–4) thanks to the dramatic progress that has led to the molecular and genetic definition of the tumor-host immune relationship. A detailed characterization of many tumor cell surface molecules that act as TAAs³ is now available (5, 6). A second cornerstone has been provided by elucidation of the way in which TAA peptides are presented to T lymphocytes in association with glycoproteins of the MHC (7, 8) and the role of dendritic cells (9) and costimulatory (10), danger (11), and cytokine (12) signals. Genetic engineering of antibody molecules (13), soluble costimulatory signals (14–16), and tumor (17) and dendritic (18) cells is used to intensify the immune response and skew it toward Th1 or Th2 reactivity. This crucial information forms the groundwork for most ongoing immunotherapy clinical trials whose clinical setting is elicitation of an immune response in a tumor-bearing patient.

Determination of which kind of patients are eligible for Phase III clinical trials is not a minor issue (19, 20). Practical and ethical constraints result in the enrollment of advanced cancer patients in Phase I trials, whereas experimental mouse data suggest that the immunity induced by specific vaccination is much more effective in the inhibition of incipient tumors than in the cure of established tumors. Elicitation of a significant response in animals with advanced tumors is exceedingly difficult, and only a minority of tumor-bearing mice are cured (21). As a tumor increases in size, it becomes refractory to immunotherapy. Its genetic instability leads to the selection of antigenic variant clones (22, 23), whereas the characteristics of its stroma (24), the peculiarity of its blood vessels (25), and its release of immunosuppressive factors (26) build up a sort of privileged site proof against immune attack.

A similar picture is emerging from Phase I immunotherapy trials. Only a few patients with established tumors display objective and in any event temporary responses (2, 3). The immunological performance status of the patients enrolled is obviously suboptimal. Most have already been treated in various ways and no longer respond to conventional therapy. Their tumor cells are selected because of their ability to escape immune reactions, and their tumor masses can suppress an immune attack. At present, immunotherapy seeks to overcome these obstacles by aggressive or combined forms of treatment (21), whereas it is becoming evident that active immunotherapy is probably not a rational option in advanced cases. Indeed, repeated failures could even jeopardize the whole of what immunotherapy is endeavoring to achieve.

However, the lethality of a tumor usually stems from the relatively small number of its cells that remain after its surgical excision and are not killed by radiotherapy and chemotherapy. The importance of this issue lies in the experimental demonstration that active immunotherapy is effective against minimum residual disease and incipient metastases and in the control of tumor recurrences (27). Early immunotherapy after a successful conventional treatment is warranted. Clinical trials suggest that patients with minimal residual disease or patients expected to present recurrences after a long interval are those for whom immunotherapy may prove really effective because it induces a prolonged tumor-free survival, if not a complete cure (1, 4). Cancer vaccines tested as single agents in advanced melanoma patients are being tested in apparently disease-free patients in combination with chemotherapy. Significant results are expected from this more rational clinical approach (28). Once the efficacy of therapeutic immunization is demonstrated, it may also be proposed as an at-home or outpatient method for the elicitation of a long-lasting immunity after the conventional management of a small tumor (17).

The Prospects of Prevention

If immunotherapy is most effective in the early stages of tumor growth, consideration can be given to an even more radical view. The use of immunological measures to prevent or forestall cancer in healthy persons has not received much attention. This is surprising because most of the experimental data obtained with transplantable tumors show that new vaccines preimmunize mice against even a poorly or apparently nonimmunogenic tumor challenge (12, 29). Furthermore, the nonspecific immunity elicited by local and systemic cytokines effectively inhibits incipient tumors until they overcome a critical threshold and become clinically evident (27). Numerous and unambiguous experimental data show that the efficacy of both nonspecific and specific immunity declines as a tumor progresses (21, 27). Whether willfully or unthinkingly, however, the evidence from preimmunization tumor challenge experiments and the cytokine-induced collapse of incipient tumors is strained to demonstrate the efficacy of immunological measures in tumor therapy and not accepted for what it really says (30), namely, that immune reactivity possesses a great preventive potential, whereas its real therapeutic efficacy against established tumors is altogether another question (17).

¹ The abbreviations used are: TAA, tumor-associated antigen; IL, interleukin; neuT, transforming HER-2/neu oncogene; neuN, nonmutated HER-2/neu proto-oncogene; Th, T helper.

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Immunoprevention of cancer would have many advantages on its side. Healthy subjects, for example, may be expected to mount a more effective and powerful response than patients who have already been treated in various ways, whereas if the target tissue is still normal or displays no more than a localized preneoplastic lesion, the chances of success should be greater than when dealing with unresetable or disseminated tumors (31). Preneoplastic lesions do not yet display genetic instability, TAA mutations, and selection of the TAA-negative clones that characterize established tumors. They should also be more permeable to immune mechanisms because their cells do not markedly remodel the extracellular matrix or produce suppressive factors, and their vessel endothelium is not yet refractory to leukocyte extravasation (32–34). Several mutations in oncogene products are required for transformation. By contrast, an alerted immune patrol would detect the initial mutations and be ready to intervene before complete transformation takes place. Although antigen(s) associated with preneoplastic lesions (as well as those for many established neoplasms) have not been yet identified, the products of mutated oncogenes are probable candidates (35). Moreover, papillomas induced by methylcholanthrene are both antigenic and antigenically distinct from each other. This suggests that TAAs characterizing subsequent progressing sarcomas are already present at the preneoplastic stage (36).

Non-specific Immunity in Cancer Prevention

The selection of not-yet patients and healthy individuals eligible for immunoprevention depends on the kind of treatment envisaged. Enhancement of non-specific immunity and specific antitumor vaccination are two possible approaches. The advantage of a non-specific antitumor response is that it can be applied directly to a broad range of individuals, irrespective of the type of TAA their foreseeable tumors may eventually express. However, it is not feasible to imagine healthy persons being treated nonspecifically for long periods. The results of the mouse experiments indicate that non-specific stimulation should thus be restricted to not-yet patients with a genetic risk of cancer (34), individuals exposed to high carcinogen doses (37), patients with a preneoplastic lesion, and patients that probably have minimal residual disease after successful conventional treatment (27). Many not-yet patients with a high risk of cancer are, in fact, being recruited in ongoing programs to screen for preneoplastic lesions or gene mutations that predispose to cancer.

Women at risk for breast cancer or with preneoplastic lesions form a category for which non-specific immunoprevention could be considered as a practical option.

However, the disclosure of a genetic risk of cancer and the presence of a preneoplastic lesion raise complex issues (38). Not a few individuals will find it difficult to cope with this information and may become deeply anxious about the possibility that they may have cancer. Routine cancer screenings, prophylactic mastectomy, and chemoprevention are all unpleasant and additionally stressful options. In some cases, the initial risk estimate will exceed the threshold for therapeutic intervention (39, 40). A “soft” immunoprevention alternative would undoubtedly be welcome.

But what has non-specific stimulation of immune reactivity to offer? A study of immunosurveillance against preneoplastic skin carcinomas suggested that it is not selective because elimination of such lesions was in no way related to their degree of malignancy (41). The extent to which non-specific stimulation can prevent the onset of cancer in cases where a risk exists has been investigated by Noguchi et al. (37) in Dr. L. J. Old’s laboratory. In their experiments, tumors were induced in BALB/c mice by s.c. injection of 3-methylcholanthrene. Delayed tumor appearance and reduced incidence were observed in mice receiving 100 ng of systemic IL-12 five days a week for 18 weeks (3 weeks on and 1 week off) during tumor latency. Secondary IFN-γ, IL-10, and tumor necrosis factor-α were evident in their sera. A high production of IFN-γ by CD8 T cells and a Th2→Th1 or Th0 shift in the cytokine secretion profile of CD4 T cells were also noted.

The ability of similar doses of IL-12 to prevent tumors when administered to mice with a genetic risk of cancer was therefore studied by us (34) in two lines of transgenic mice expressing the rat HER-2/neu oncogene under the transcriptional control of mouse mammary tumor virus (34). Female BALB-neuT (H-2b) mice carrying the transforming HER-2/neu oncogene show no morphological abnormalities of the mammary gland until 3 weeks of age. They then progress through atypical hyperplasia to in situ lobular carcinoma, and at 33 weeks of age, all 10 mammary glands display invasive carcinomas. In adult FVB-neuN (H-2b) mice carrying the HER-2/neu proto-oncogene, neoplastic progression is less impetuous, as shown by a longer latency (38–49 weeks) and a lower tumor multiplicity (mean, 2.6 tumors/mouse). Treatment with IL-12 (five daily i.p. injections; 1 week on and 3 weeks off; the first course with 50 ng IL-12/day and the following courses with 100 ng IL-12/day) begun at 2 weeks of age in BALB-neuT mice and at the 21 weeks of age in FVB-neuN mice markedly delayed tumor onset and reduced tumor multiplicity. In both lines, tumor inhibition was associated with deficient peri- and intratumoral angiogenesis, infiltration of reactive cells, production of proinflammatory cytokines, and inducible nitric oxide synthetase activation.

We next set out to determine the stage at which administration of IL-12 is most effective. Was it simply a preventive measure in still healthy animals or could it also be of benefit once overt preneoplastic lesions are diagnosed? Groups of BALB-neuT and FVB-neuN mice received IL-12 at progressive times during carcinogenesis (42). In both lines, IL-12 was particularly effective in inhibiting the progression from hyperplasia to in situ and invasive carcinoma, i.e., at the time of the angiogenic switch. Its antiangiogenic effect is markedly evident on the fragile capillaries sprouted during this switch. Late administration was poorly effective in both mouse lines, presumably because the mature and differentiated blood vessels of more advanced lesions are less sensitive to IL-12-induced inhibition. However, the antitumor action of IL-12 is not confined to its indirect influence on endothelial cells. Directly or through secondary cytokines, it triggers lytic activity and mediator release from a variety of tumor-infiltrating leukocytes and thus counters the continuous generation of transformed cells. Its efficacy, in fact, probably rests on the sum of its activities, and not simply on the blocking of tumor angiogenesis, important as this may well be (27). These experiments also show that lifelong administration is not required for genetically determined cancers with a long natural history. Precise definition of the carcinogenic events may allow preventive treatments to be performed only during a critical stage of the long carcinogenic progression.

The HER-2/neu oncogene is expressed in a substantial proportion of human mammary carcinomas. The close resemblance of the progression of mammary carcinogenesis in HER-2/neu transgenic mice to that in women suggests that the administration of nontoxic recombinant IL-12 regimens may be a significant prophylactic strategy. The direct proportionality between the length of carcinogenesis progression and the efficacy of IL-12 observed in these models suggests that stimulation of non-specific immunity could be envisaged as an effective, preventive way of slowing human carcinomas (30).

The principles illustrated by these models are clear. The extent to which they reflect the situation in humans must obviously be established in clinical trials, especially because the immunological weight of IL-12 may not be the same in mouse and human tumors. In the meantime, further evidence that cytokine-elicited immunity can prevent tumor progression is provided by a randomized multicenter Phase III trial with low, nontoxic doses of IL-2 injected locally.
Patients with resectable $T_{3a}$-$N_{0a}$-$M$ squamous cell carcinomas of the head and neck receiving supplemental IL-2 before and after surgery displayed a significantly extended disease-free interval as compared with those treated only with conventional therapy.4

**Specific Antitumor Vaccination of Persons at High Risk of Cancer**

Specific vaccination of persons at risk and healthy individuals constitutes a very different scenario. Characterization of specific gene alterations or detection of preneoplastic lesions may indicate which organ and tissue are at risk. In a few cases, more precise information may show which oncogene product will probably be overexpressed or expressed in an altered form and allow vaccination against a single, specific TAA. Molecular characterization of altered gene products predictably destined to become TAAs will be the first step toward the engineering of selective vaccines (43). Otherwise, the patient should be vaccinated against the TAA most commonly expressed by the tumors foreseeable in a given organ.

Many new antitumor vaccines that induce an effective resistance to subsequent tumor challenge and inhibit minimal residual disease are already available (12, 29). The question of whether specific immunization can be successful once a cell population has been subjected to the initial carcinogenic hit has rarely been examined experimentally. However, it can be plausibly suggested that cytokines and more conventional adjuvants could induce an effective immune response against ignored or fully tolerated antigens. The specific immunity elicited in mice transgenic for rat Her-2/neu is a sign that specific vaccination induces strong immune responses against such antigens and may thus inhibit oncogenesis and extend survival (44–46).

**General Antitumor Vaccination**

One can also envisage the even wider application of antitumor vaccines to prevent tumors in the general population, as is done for infectious diseases. This point considers the possibility of preventing the onset of cancers related to an infectious agent by vaccination against the agent itself. This approach is applicable to a sizeable proportion of diverse human tumors including cervical carcinoma (human papillomaviruses), hepatocellular carcinoma (hepatitis B and C viruses), and Burkitt’s lymphoma (EBV). A significant impact of hepatitis B vaccination on the incidence of hepatocellular carcinoma has already been reported (47), and promising results being obtained in preclinical models of papillomavirus oncogenesis (48) suggest that human vaccination will eventually be able to prevent cervical carcinoma (49).

Molecular and genetic data suggest that human TAAs identified as targets of CTLs (5) or by the SEREX technique (6) can be divided into classes. One class consists of tumor-specific antigens coded by genes expressed by tumors but not by normal cells, with the exception of male germinal cells. However, because these cells do not express MHC glycoproteins, they do not present peptides from the protein products of these genes on their surface. The use of these antigens in preventive vaccination is interesting because their number seems not to be endless, and they are shared by histologically distinct tumors arising in different organs. Furthermore, the telomerase catalytic subunit is markedly activated in more than 85% of human tumors, whereas it is silent in normal tissues and thus constitutes a sort of universal TAA (50). The second class of antigens derived from point mutations looks less interesting for general vaccination because they are unique for a given tumor, and their expression by a foreseeable tumor is poorly predictable. Nevertheless, in some cases, oncogenes and oncosuppressor genes display a narrow spectrum of mutations (e.g., RAS; Ref. 51). In addition, chromosome translocations that give rise to fusion proteins display a relatively constant pattern of junction between the two genes.

Another class comprises antigens that are also expressed by normal cells of the same differentiation lineage. Immune reactions elicited against them could be coupled with the induction of an autoimmune disease. An additional class is formed of molecules expressed by normal cells and overexpressed by neoplastic cells. Here, too, there is a risk of inducing autoimmune reactions. In fact, once the immunological ignorance or tolerance against these antigens is overcome, effector mechanisms endowed with a lower threshold of activation may destroy both normal and neoplastic cells. However, experimental data from variously immunized mice did not disclose major autoimmune lesions as a side effect of vaccination with these antigens. On the contrary, a specific immune reaction often affected tumor cells overexpressing the target TAA and spared normal tissues where TAA was expressed at a much lower level (52).

Because many TAAs are shared by a variety of tumors, preventive immunization against most common human cancers with not many more than 20 TAAs would seem conceivable. A possible list would include the infectious agents mentioned earlier, mutated oncogenes, telomerase catalytic subunit, and antigen of the MAGE family. However, the erratic boundary between tumor immunity and autoimmunity (53) means that the risk of inducing an autoimmune disease is a major concern. This risk would be much weightier in the vaccination of healthy individuals as opposed to individuals at risk, where the scales of risk-benefit are markedly biased by the higher risk of cancer and the consequent shorter life expectancy. A further warning is related to “epitope spreading” (54). Several data in animal models show that immune responses to a few self-determinants shift drift and diversify with time and include other epitopes of the same proteins or other proteins.

The planning of vaccines à la carte by genetic engineering may be a way to selectively trigger reaction mechanisms that ignore cells that express a low density of the target antigens or are less prone to induce a widespread autoimmunity. Consideration must also be given to the balance between the kind of potential autoimmunity and the degree of lethality of the possible tumor. Autoimmune vitiligo, for example, would be a relatively small price to pay for protection against melanoma, whereas in other situations, such as the prevention of bowel tumors, the risk of more severe autoimmune diseases would demand a careful approach. Experimental studies should address this issue in detail.

Another limitation to be carefully weighed is the constraint imposed by the polymorphism of MHC glycoproteins and the repertoire of peptides presented. Different peptides would need to be prepared to fit in the polymorphic peptide-binding clefts of the many MHC class I and II glycoproteins. It is predictable that certain TAAs will have a restricted usage, and a few individuals will not be easily vaccinated.

Elicitation of a “surgical” immune response ablating only cells that express a specific antigen is probably impossible. This does not mean, however, that individualized vaccines are a strict necessity. Vaccines have to be reprocessed by the immune system of the host. Therefore, in many instances, the presence of inappropriate antigens, for example, allogeneic MHC molecules, could result in the establishment of a polyclonal T-cell activator that would favor and not hamper the induction of a restricted, peptide-specific immune response (43, 44, 55).

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Conclusions

The cardinal prerequisite of preventive medicine is the Hippocratic “do no harm”: “primum non nocere” (56). Prolonged nonspecific and specific immune stimulation of persons at risk and the general population is indeed not free from uncertainty, although identification of the steps of tumor progression most susceptible to the immune mechanisms elicited could drastically reduce the stimulation period (42).

However, as stressed in a recent report on cancer chemoprevention (56), failure to intervene when a disease as diffuse and dramatic as cancer can be prevented can also be viewed as harmful. The idea that it is not appropriate to treat healthy persons with cytokines or with antitumor vaccines because of the risks involved will hopefully be shown to be a misconception. An equal or even higher risk of inducing autoimmune complications is associated with many antimicrobial vaccines. Fortunately, they came into use before this risk was perceived. Had it otherwise, their employment would have been much more strongly opposed, and many more persons would have died.

In conclusion, immunoprevention of cancer seems a distant but plausible prospect. Experimental elucidation of its critical issues could provide essential information for its application in humans. Prevention itself would provide a fresh and perhaps conclusive way of winning the long-lasting war against cancer. Manipulation of the immune response to prevent cancer could soon lead to the realization of a notion that has deep roots in the history of immunology (57).

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References


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