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Exploiting liver immunity for the prevention of hepatic metastases

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Over the last decade immunotherapy has progressively gained a significant clinical interest for cancer treatment.

The actual benefit of *active immunotherapy*, namely cancer vaccines, has been repeatedly claimed as effective in several settings and prompted prospective investigations (phase III clinical trials) currently ongoing in patients with prostate carcinoma, melanoma, and lung cancer, while the first vaccine for the treatment of metastatic prostate carcinoma has been recently approved by the FDA [1,2].

Adoptive immunotherapy, based on the administration of antibodies (Ab) targeting tumor receptors or molecules expressed in the microenvironment, has also entered clinical practice in several cancers such as breast carcinoma, colorectal cancer, and haematological tumors [3], whereas adoptive transfer of *ex vivo* activated tumor-specific T cells has shown dramatic efficacy in metastatic melanoma [4], a known benchmark-tumor for immune manipulations both in animal and human models.

Ab-therapy of cancer was initially conceived to directly eliminate tumor cells by interfering with the activity of either receptors involved in cancer cell proliferation (e.g. members of the HER family) or molecules regulating tumor blood supply (such in the case of anti-VEGF Ab) [5]. However, the clinical efficacy of broadly used Ab was subsequently found to rely, at least in part, on the contribution of the immune system through the activation of antibody-dependent cell cytotoxicity (ADCC) mediated by cellular components of *innate immunity* [5,6]. NK cells, monocytes, macrophages, and granulocytes can indeed bind to Ab thanks to the expression of specific receptors for the invariant portion of this molecule (the so called Fc portion) and be recruited to attack target cells, hence contributing to the therapeutic potential of Ab-based therapies. Evidence proving such mechanism has been collected in preclinical studies, showing that anti-tumor antibodies, like trastuzumab, have reduced activity when administered in FcR knock-out mice or used in a FcR-binding defective form. Similarly in clinical setting, breast cancer trials have reported immune infiltrate in cancer lesions of Ab-treated patients, and better efficacy in subjects expressing defined FcR genotypes [6]. More recently, the anti-tumor activity of such strategy has been also potentiated through bi-specific engineered single-chain Ab designed to tether cytotoxic T lymphocytes to cancer cells, an approach associated with significant responses in patients with non-Hodgkin's lymphoma [7].

Most human cancers are currently acknowledged to be the target of some type of immune recognition; among them colorectal carcinoma (CRC) has been unequivocally proven to be under the control of tumor immunity, particularly in the early phases of its development. Indeed the presence of T cells with memory phenotype infiltrating primary tumor lesions showed a favourable prognostic impact in patients with CRC, suggesting how host immune surveillance may initially impact the course of the disease [8]. However, with cancer progression, immunological defects targeting both innate and adaptive immunity tend to accumulate, blunting initial defences for promoting an immunosuppressive pro-tumorigenic environment [9,10]. In fact, as a consequence of intrinsic tumor pathways or chronic inflammation, dendritic cells and macrophage with altered stimulating activity, together with regulatory T cells and myeloid-derived suppressor cells, expand *in vivo* and exert potent inhibitory effects on anti-tumor immunity [10].

Because of this, any form of immunotherapy relying on the contribution of the host immune system finds its ideal application in early disease or in the adjuvant setting, when the tumor burden is still microscopic and the host's immune function is mostly preserved.

Adjuvant immunotherapy to prevent hepatic metastases from CRC is actually the setting chosen by van der Bij and collaborators, [11] who report, in the present issue, the successful use of a specific anti-tumor Ab to inhibit, in a rat model, the growth of metastatic CRC cells injected into the liver through the portal system. This experimental setting has some limitations with respect to real patients in which CRC cells spill into the portal system from a primary intestinal tumor site that owns variable predisposition to venous invasion. However, in the absence of the primary tumor component, this study is actually reminiscent of two crucial conditions that may affect the outcome of patients with CRC: (1) the fact that microscopic tumor cell deposits in the liver precede the development of an unknown proportion of such cells into clinically established metastases, and (2) the potential pro-tumorigenic effect of surgical intervention, possibly related to the production by healing tissues of growth factors, angiogenesis mediators, inflammatory cytokines, and chemokines [12,13], with consequent advocacy of minimally invasive techniques in removing primary colorectal cancer sites.

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The study of van der Bij et al. depicts the possibility of preventing liver metastases by post-operative (adjuvant) administration of a tumor-specific monoclonal Ab, whose potent prophylactic effect was not directly mediated by the Ab itself but required the activation of local immune effectors, namely Kupffer cells (KC) and possibly monocytes.

One of the innovative aspects of the present work is indeed the idea of specifically exploiting resident components of the *innate immunity* to promote cancer cell clearance in the liver. Indeed, immunologists consider the liver a lymphoid organ [14] hosting a wide variety of immune cells, particularly KC which are liver-confined macrophages with prompt phagocytic and cytotoxic activity [15]. KC are acknowledged to provide a second line of defense in mucosal immunity by eliminating invasive microorganisms entering through the portal circulation and thus preventing disease thanks to their expression of different immune receptors (such as for instance FcR) [16]. It is hence conceivable that they may also act as a sentinel in eliminating tumor cells if appropriately stimulated, as clearly suggested by van der Bij and collaborators. Although the expression of immune-related receptors surely differs in rodents and humans [17], KC may undoubtedly represent a useful ally for reducing the incidence of liver metastases in CRC patients, pending the identification of the optimal stimuli for rescuing their anti-tumor activity.

Interestingly, the liver is also rich in resident NK cells [14], the immune subset mostly involved in ADCC in humans. Tumor-specific Ab capable of engaging both KC and NK cells by binding to common receptors and possibly promoting a positive cross-talk (such as toll-like receptor agonists [18]) should help maximizing the local effect on tumor control.

Additional key points to be addressed in case of clinical applications of the proposed strategy are: the choice of antibodies able to bind efficiently and specifically to CRC cells rather than normal cells; the identification of tumor antigens possibly playing a role in regulating tumor cell proliferation, to be targeted by these Ab, and the homing capacity of the chosen Ab that could be potentiated, as in the described study, by intra-portal surgical routing.

Besides the family of epidermal growth factor receptors, other appealing antigens to be targeted are represented by mucins, broadly expressed [19], and already tested as target of spontaneously or vaccine-induced immune responses in CRC patients [3]. Also the newly identified CP1 (cancer placenta 1) is highly expressed in CRC cells and is able to elicit both humoral and cellular immune responses correlated with survival in CRC patients [20].

If the innate liver immunity could be triggered by Ab to control growth of metastatic tumor deposits into the liver, activation of adaptive immunity through the administration of a cancer vaccine could provide the additional advantage of inducing a protective immunological memory for long-term and systemic disease control, a feature that would not be directly induced by Ab-therapy. Indeed, we have shown that an anti-tumor vaccine (composed by heat shock protein 96 extracted from liver metastases) can significantly reduced the recurrence rate and prolong survival in CRC patients mounting a CD8-mediated tumor-specific response to immunization [21,22]. Cancer vaccines could hence be administered in combination with Ab for more full-fledged immune mediated tumor control. With liver being an organ where potent CD8+ T cell priming can occur, cancer vaccines could also be administered locally to promote activation of resident tumor-specific T cells [14].

More extensive studies on the role of resident liver immunity in controlling local tumor growth should thus be encouraged, together with a fine analysis of the immunological features of resident KC, macrophages, and NK cells in the liver. Most importantly, translation of the achieved results into the clinical setting should be accelerated, to test whether adjuvant immunotherapy, possibly combining tumor-specific Ab and vaccines, can significantly impact the course of disease in CRC patients.

Conflict of Interest

The authors declared that they have no disclosures regarding funding or conflicts of interest with respect to this manuscript.

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