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Commentary

Rejection antigens in chemically induced tumors

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The paper of Ikeda *et al.* (1) throws new light on an old problem. Four decades ago chemically induced mouse and rat tumors were found to elicit tumor-specific rejection reactions in syngeneic and even in autochtonous hosts. The tumor-specific transplantation antigens (TSTAs) that were defined by the rejection response were individually distinct, with no consistent cross-reactions between different tumors of the same type. In contrast, virus-induced tumors carried group-specific TSTAs, common for all tumors induced by the same virus. Immunization against both types of TSTA protected the host only against small or moderate cell doses. Resistance could be transferred with lymphocytes, but not with serum (2).

During the following decades, much progress was made in defining the nature of the virus-induced TSTAs. With polyoma and simian virus 40 in mice and Epstein–Barr virus in humans as paradigmatic examples, major histocompatibility complex class I-associated peptides derived from virally encoded, transformation-associated proteins were identified as being responsible for the immunogenicity and immunosensitivity of these and other virally induced tumors.

The nature of the TSTAs in the chemically induced tumors and the reasons for their diversity remained enigmatic. The work of Ikeda *et al*. (1) is an important advance. Following the methodology developed by Boon and his colleagues (3), they used a line of cytotoxic T cells (CTLs), specific for a methylcholanthrene (MC)-induced sarcoma cell. CTLs against MC-induced sarcomas have been established previously (4), but the target antigen has not been identified. Ikeda *et al*. (1) screened a cDNA expression library prepared from the target tumor. The gene encoding the protein recognized by the CTLs was identified as a mitogen-activated protein kinase (MAPK) mutant. The target peptide differed from its normal counterpart by a single amino acid substitution. With the help of interleukin-12, the mutant peptide could elicit the tumor-specific rejection response that served as the point of departure.

This spectacular success in an area that was at a total standstill for so long raises many old and new questions. The authors suggest that their inability to select nonimmunogenic variants by exposing the sarcoma cells to the specific CTLs *in vitro*, or by passaging them through preimmunized mice, may indicate that the mutated MAPK gene is essential for the neoplastic behavior of the target cell. The same argument has been used previously for polyoma-induced tumors (5). The subsequent definition of polyoma TSTA as T antigen-derived peptides was consistent with this idea. Ikeda *et al*. (1) could not prove their point by *in vitro* transformation experiments, however. It may be also noted that Dudley and Roopenian (6) reported successful CTL-mediated immunoselection against MC-induced sarcomas, generating both major histocompatibility complex class I and unique tumor antigen loss variants.

Will the indefinitely diverse TSTAs of the MC-induced tumors turn out to be different mutants of the same family of molecules? Or does MC and other aromatic hydrocarbons that generate tumors with a similarly distinct antigenicity act on the "let hundred flowers bloom" principle, due to their combined mutagenic and immunosuppressive effect? Do the tumors they produce represent a spectrum of potentially immunogenic clones that are normally rejected (7–9)? The latter possibility is consistent with the finding that chemically induced primary tumors that arose after a short latency period were more immunogenic than their more delayed counterparts, indicating that immunoselection was at work (10, 11).

Have mutant proteins that can be suspected on reasonable grounds to contribute to the tumorigenic process made themselves known as tumor antigens with a rejection eliciting potential in other systems? There are only a few reports in this category. Using Boon's technology (12), Wolfel *et al*. identified a melanoma-specific CTL clone that targeted a CDK4 mutant that was no longer able to bind the p16 tumor-suppressor protein. They suggested that the CDK4 mutant, which could be identified in one additional melanoma among 28 analyzed, can create a tumor-specific antigen and can also disrupt the cell cycle regulation exerted by the p16 tumor suppressor.

Boon's and van der Bruggen's own group (S. Mandruzzato, S. Brasseur, G. Andry, T. Boon, and P. van der Bruggen, personal communication) have recently identified a mutated FLICE protein, presented as a CTL-recognized tumor antigen, in a human squamous cell carcinoma of the oral cavity. The protease product of this gene, caspase-gamma, is required for the induction of apoptosis through the Fas and TNRF1 receptors. It was suggested that the mutation may have increased the resistance of the carcinoma cells to apoptosis.

It is somewhat surprising that none of the frequently mutated oncogenes or tumor suppressor genes, such as ras or p53, have been picked up by the Boon technology in human or animal tumors as natural CTL targets. Synthetic ras and p53 mutant peptides could be used to generate cytotoxic and/or rejection responses against tumors that carried the corresponding mutant genes (13, 14), but no similar responses have been encountered in experiments based on the detection of T cell sensitization against tumor-associated antigens in mixed lymphocyte tumor cell cultures. Could this explain some of the idiosyncratic features of the mutated or otherwise activated oncogenes and tumor suppressor genes? I am referring to the fact that some proteins within complex signal transducing or growth cycle regulatory chains frequently contribute to the tumorigenic process, whereas their nearest upstream or downstream neighbors, which might be expected to do the same, fail to appear on the list of known cancer-related genes. Could this be due, at least in part, to the rejection-inducing potential of their mutants? Is the presently known oncogene/tumor suppressor gene spectrum biased by an "immunological filter" of this kind? Would a highly immunosuppressive carcinogen, like MC, permit the mutants of as yet unidentified oncogenes to slip through that filter? Is the MAPK the first example in that category? If so, the identification of further rejection-inducing immunogens expressed on MC-induced tumors may turn out to be highly rewarding.

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