Tumor-Specific Antigens and Tumor-Specific Mutant Proteins in Mouse and Man *

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A. Introduction

The modern era of cancer immunology began with the discovery that inbred mice could be immunized against cancers that had been induced by chemical carcinogens such as the polycyclic hydrocarbon methylcholanthrene (MCA) [1–4]. Particularly, studies of Prehn and Main in 1957 made it highly unlikely that the antigens on the cancers were also widely expressed on normal tissue. It was shown that normal tissue of the host from which the tumor had been isolated did not immunize the recipient to reject the tumor challenge; furthermore, mice immunized against the tumor still accepted normal skin grafts from the mouse of tumor origin. Thus, these antigens were seemingly tumor specific. Another important aim of the experiments using MCA-induced murine tumors was the search for antigens that were tumor specific as well as shared among different independently induced cancers. The identification of such antigens would allow the same antigen to be utilized for the therapy and diagnosis of different types of cancers occurring in different individuals. The existence of such antigens would have great significance in medical praxis. However, very extensive transplantation experiments showed that the tumor-specific rejection antigens on these cancers were unique, i.e., individually specific for a particular tumor even when compared to other tumors of the same histologic type induced in the same organ system with the same carcinogen in supposedly genetically identical mice. In fact, careful studies searching for cross-reaction among ten tumors expressing unique antigens showed no repeatable protective immunity except when immunization and challenge involved the same tumor [5]. Thus, it appears from these studies that the antigenic repertoire is, in fact, very large. Tumors induced with other chemical and physical carcinogens and even spontaneous cancers also display unique (individually specific) antigens that can elicit tumor rejection [6–11]. Finally, a single cancer cell may display multiple independent unique antigens, so that the diversity of unique antigens may be greater than previously anticipated [12].

B. Genetic Origin of Murine Unique Tumor Antigens

The seemingly endless diversity of unique tumor antigens on experimentally induced and spontaneous cancers has stimulated the interest of many immunologists. Burnet, for example, postulated that the unique antigens might be the result of clonal expansion of single cells expressing the particular (preexistent) antigen [13]. This situation would be similar to the idiotype of B- and T-cell malignancies that are individually distinct and are immunogenic in the host of origin [14, 15]. The nonmalignant clone carrying the idiotype is, under normal circumstances, present in too low a frequency to be detected by the immune system or the

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* This work was supported by grants CA-19266, CA-22677, and CA-37156 from the National Institutes of Health

scientist trying to prove the absolute restriction of the antigen to malignant cells. Burnet suggested that gene families known to allow enormous antigenic diversity, such as the receptors for antigens on T and B cells or MHC class I antigens, could represent the genes encoding tumor antigens [13]. In fact, certain experiments pointed at the possibility that immunoglobulin genes or MHC class I genes can encode unique tumor-specific rejection antigens [16]. The question of whether amplification of preexistent normal clonal antigens is the basis for the uniqueness of tumor-specific antigens has been addressed experimentally. In two such studies, a nonmalignant fibroblast line was cloned, then expanded, and subclones were malignantly transformed [17, 18]. Immunological studies indicated that all had individually distinct antigens even though all tumors had been derived from the same precursor cell. At face value, these experiments seem to indicate that the appearance of the antigens followed the carcinogen exposure and that these are, therefore, new antigens or neoantigens that were not previously expressed on the precursor cell. However, normal cells can generate considerable diversity of surface molecules during clonal expansion from a single precursor [19, 20], and the transformation event caused by the carcinogen may simply fix a particular antigenic phenotype [21]. Alternatively, it is possible that normal previously nonexpressed genes are randomly activated by the carcinogen [22]. Obviously both mechanisms could produce considerable antigenic diversity with apparent tumor specificity even though these antigens are expressed on normal cells. Sometimes only restricted populations of normal cells express these antigens, so the fact that they are not tumor specific may be difficult to recognize since the appropriate control cells expressing this antigen may not have been tested [22]. Together, the previous experiments cannot prove the possibility that the so-called tumor-specific antigens are tumor specific in the strictest sense since they might be encoded by normal genes and even be expressed on an unrecognized normal cell population.

C. Are Unique Tumor Antigens Encoded by Tumor-Specific Mutations?

Since most, if not all, carcinogens are mutagens, it appears quite logical to hypothesize that tumor-specific antigens may commonly arise from tumor-specific mutations of structural genes. The extreme uniqueness of transplantation antigens induced by chemical carcinogens would be consistent with the fact that mutagenic chemicals randomly affect genes. However, to date there is no genetic evidence that a cancer-specific mutation and not normal genes encoded in the germline encode unique tumor antigens. Recent work in animal tumors led to the development of cytotoxic T-lymphocyte (CTL) and antibody probes that can be used to unravel the genetic origins of unique tumor antigens. However, there are serious questions whether previously isolated tumors can be used for a meaningful genetic analysis of the origin of unique antigens, since none of the previously generated tumors were isolated along with nonmalignant control cells and DNA. Without such controls one cannot prove that a particular abnormal gene was not already present in normal DNA of the host in which the tumor originated. This is particularly relevant since subtle germline mutations, residual heterozygosity, contaminations of the strain of tumor origin during breeding [23] would easily be distinguished from tumor-specific mutations if autochthonous normal DNA was available for each tumor analyzed [16]. Our laboratory has previously used ultraviolet light (UV)-induced murine skin tumors as an experimental model to study the host's immune responses against a cancer [24, 25]. These tumors often exhibit such a strong immunogenicity that they are rejected by syngeneic animals. We recently generated a new series of UV-induced

tumors [32]; these tumors were isolated with all necessary controls, such as cells and DNA from normal tissues of each tumor-bearing animal. This material should enable us to unravel the genetic origin of unique tumor antigens and finally answer the question of whether these antigens are tumor specific in the strictest sense, in that they are encoded by tumor-specific genes not present in normal somatic cells of the host of tumor origin.

D. Do Tumor-Specific Mutant Proteins Encode Tumor-Specific Antigens?

It must be expected that chemical and physical carcinogens mutate intracellular as well as surface proteins. Many, or most, of these mutations are probably a disadvantage to the cell and are, therefore, selected against during the clonal evolution of cancer [16]. In contrast, specific mutational changes that favor the malignant process would be retained. An example is a highly selected point mutation caused by the chemical carcinogen nitrosomethylurea in the cellular *ras* oncogenes [26]. This mutation favors malignant growth and is, therefore, found regularly in certain tumors, such as mammary tumors induced by this carcinogen. Other examples of mutations leading to fusion of exons between distinct genes that are brought together by tumor-specific translocations are found in certain types of human leukemias ([27–30], also see J.D. Rowley, this volume). Thus, fusion between the *BCR* and *ABL* genes leads to several types of fusion proteins that must clearly be expected to generate a new antigenicity. Since these fusion genes caused by the translocations are not observed in normal cells, one can assume that these genes may well encode truly tumor-specific antigens. The mutant *ras* genes, as well as the *BCR-ABL* fusion genes, encode intracellular proteins. Until recent years, it was postulated by immunologists that CTL could only recognize cell surface proteins. However, previous and recent evidence demonstrating CTL recognition of the nuclear SV40 virus T antigen and influenza virus nuclear protein made it clear that intracellular proteins are indeed recognized by CTL (for review see [31]). The explanation for this enigmatic finding is that CTL can recognize peptides of enzymatically cleaved antigens which are then "expressed" on the cell surface in association with MHC class I molecules.

E. Conclusions

Although we lack conclusive evidence, it is certainly possible that tumor-specific mutant proteins can be recognized by CTL or helper T cells as tumor-specific antigens. Interestingly, mutant genes such as *BCR-ABL* represent mutations that are shared by leukemias of the same type but independently induced in different patients. Thus, these changes represent common or shared tumor-specific mutations that may encode yet common tumor-specific antigens in man. This is important since the search for common yet tumor-specific antigens in experimental tumors has been without convincing success. At present, we do not know how regularly tumor-specific mutant proteins are found in human cancer cells, or whether they indeed encode tumor antigens that can be exploited therapeutically and diagnostically. However, it is likely that more tumor-specific mutant proteins will be discovered in human cancers in the future and that cancer development as a multistep process is probably dependent upon several rather than a single mutational event. Certainly, several of these mutations, such as the *BCR-ABL* fusion gene, may be essential for maintaining the malignant phenotype. Such mutant proteins, if they act as tumor-specific antigens would be ideal targets since the cancer cell could not escape therapy directed at this target by gene loss or down-regulation. Thus, discovery of these mutant proteins that are truly tumor specific and genetically de-

fined needs the most serious evaluation by tumor immunologists.

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