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Derepressed Alloantigen on Transplacentally Induced Lung Tumor Coded for by H-2 Linked Gene

Abstract. *A transplacentally induced lung tumor of strain C3Hf mice grows progressively when transplanted to (C3Hf × A)F_1 hybrid mice but not when transplanted to C3Hf recipients. Progressive tumor growth occurs in [(C3Hf × A)F_1 × C3Hf] backcross mice inheriting the H-2^a haplotype from the F_1 parent. Furthermore, radioresistant immunity to the lung tumor can be induced in C3Hf mice by immunization with normal tissue of B10.A and B10.A(2R) but not of B10 or B10.A(5R) strain mice.*

According to the concept of immune surveillance, a major function of the immune system is the detection and elimination of nascent tumors (*1*). This concept requires that nascent tumors express cell surface components distinguishable immunologically from components expressed on normal cells. Progressive growth of a tumor induced in an adult animal would reflect an escape from immune surveillance possibly because of a change in the tumor-associated surface antigens (TASA) expressed by the nascent tumor. The TASA on progressively growing tumors induced in adult animals might not be equivalent therefore to the TASA on nascent tumors subject to successful immune surveillance (*2*).

We have addressed this problem by analyzing the TASA in tumors induced transplacentally at a time prior to the maturation of immune competence. Fetal mice aged 13 to 17 days that are exposed transplacentally to the rapidly acting carcinogen 1-ethyl-1-nitrosourea (ENU) develop a high incidence of malignant lung tumors (*3*). Since microscopic lung tumors of mice treated transplacentally with the related carcinogen, urethane, are demonstrable at birth (*4*), it is likely that tumors induced by the more potent carcinogen ENU are also present at birth. The TASA on such tumors might therefore be recognized as self antigens by the maturing immune system. These TASA would, however, be considered as foreign antigens when the tumors were transplanted into syngeneic recipients. It is interesting, therefore, that a transplacentally induced lung tumor of a C3Hf mouse, designated 85, grows poorly when inoculated into C3Hf recipients but grows rapidly when inoculated into (C3Hf × A)F_1 hybrid recipients (*2*, *5*). The resistance of the C3Hf mice to 85 tumor growth is immunologically mediated and can be overcome by prior x-irradiation provided that the mice are not previously immunized against the lung tumor. The successful growth of lung tumor 85 in (C3Hf × A)F_1 hybrid mice is due to the expression on lung tumor 85 of a TASA which exists as a normal tissue component in strain A mice. Thus it is possible to elicit specific radioresistant immunity to lung tumor 85 in C3Hf mice by immunization with normal tissues of strain A mice (*2*). The tumor-associated alloantigen is not expressed in normal tissues of either C3Hf, C57BL/6, or DBA/2 mice (*2*) although, as will be documented elsewhere, at least 10 of 50 transplacentally induced lung tumors of these strains express the strain A–associated tissue alloantigen (*6*). Here, we report that the alloantigen expressed as a TASA on lung tumor 85 of C3Hf mice is coded for by a gene linked to the K end of the H-2 major histocompatibility complex.

The growth of lung tumor 85 in [(C3Hf × A)F_1 × C3Hf] backcross mice (*7*) was determined by inoculating the mice intradermally with 10^5 tumor cells. Of 48 backcross mice, progressive tumor growth (mean diameter in excess of 10 mm at 28 days) occurred in 23 mice,

Table 1. Residual H-2^a cytotoxicity of D-28 antiserum absorbed with spleen cells of [(C3Hf × A)F_1 × C3Hf] backcross mice tested for susceptibility to the growth of lung tumor 85. Spleens were removed from the two groups of mice and stored at −80°C. Each spleen was subsequently thawed and teased to yield a tissue suspension. The tissues were tested for their capacity to absorb cytotoxic activity from a 1 : 50 dilution of D-28 antiserum. The absorbed portions of antiserum were tested for residual cytotoxicity against spleen cells of strain A mice. The unabsorbed serum lysed an average of 88 percent of the spleen cells.

Source of spleen cells	No. of spleens tested	Residual cytotoxicity	
		Mean	Range
Tumor-susceptible backcross mice	23	17.4	9 to 41
Tumor-resistant backcross mice	25	86.2	78 to 91

Table 2. Radioresistant immunity to lung tumor 85 induced by immunization with normal tissue of allogeneic mice. In experiment A, C3Hf mice were immunized with lung tissue, while in experiment B, (C3Hf × DBA/2)F_1 mice were immunized with liver tissue from the donor strains.

Donor strain of tissue used for immunization	Tumor growth in x-irradiated mice	
	Proportion of mice with tumors	Mean tumor diameter (mm)
	Experiment A	
None	7/7	22.1
B10	13/13	21.7
B10.A	2/9	2.0
	Experiment B	
None	10/10	14.1
B10.A	0/6	0.0
B10.A(2R)	0/13	0.0
B10.A(5R)	12/12	16.3

while tumor growths of 0 to 3 mm occurred in 25 mice. All the mice were tested for the capacity of their spleen cells to absorb the cytotoxic activity of an antiserum, designated D-28, which is reactive with cells of the H-2^a haplotype (strain A) but not with cells of H-2^k mice (C3Hf) (*8*, *9*). The results are depicted in Table 1. Spleen cells from [(C3Hf × A)F_1 × C3Hf] backcross mice permissive for tumor growth absorbed cytotoxicity from the D-28 antiserum and therefore these mice must have inherited the H-2^a haplotype. Spleen cells from tumor resistant [(C3Hf × A)F_1 × C3Hf] backcross mice failed to reduce significantly the cytotoxicity of the D-28 antiserum and therefore these mice must not have inherited the H-2^a haplotype. These data indicate that the differential growth of lung tumor 85 in C3Hf and (C3Hf × A)F_1 hybrid mice is due to a single genetic locus and that the locus is linked to the locus controlling the expression of the H-2^a haplotype in strain A mice.

To test further the linkage of the TASA to the H-2 complex, C3Hf mice were immunized with lung tissue from B10 mice (H-2^b) and B10.A mice (H-2^a) prior to x-irradiation and challenge with 10^5 cells from lung tumor 85. As shown in Table 2, radioresistant immunity to lung tumor 85 was induced by lung tissue from B10.A mice, but not by lung tissue of the congenic B10 mice. In similar experiments, (C3Hf × DBA/2)F_1 hybrid mice were immunized with normal tissue from either B10.A (2R) or B10.A (5R) recombinant mice and tested for radioresistant immunity to lung tumor 85. The 2R recombinant expresses the K region of the H-2^a haplotype while the 5R expresses the D region of the H-2^a haplotype (*9*). As shown in Table 2, immunity to lung tumor 85 was achieved by immunization with tissue from the 2R but not the 5R recombinant strain. This result indicates that the gene coding for the alloantigen expressed on tumor cell 85 is linked to the K end of the H-2 histocompatibility complex. Since the locus controlling the expression of the TL alloantigen (a thymus leukemia–associated antigen) is linked to the D end of the H-2 complex (*9*), the lung tumor–associated–antigen is not coded for by a TL-linked gene. Similarly, the type C viral alloantigen G_{IX} can be excluded, since in those strains in which the G_{IX} locus appears to be linked to H-2, it has been localized distal to the D end of the H-2 locus (*10*). The K ends of the H-2^a and H-2^k haplotypes are thought to code for identical serologically defined alloantigens (*9*, *11*). The data reported here indicate that important differences do exist in the region of the K end of the H-2 complex of A and C3Hf mice and emphasize the importance of genes which are linked to, or are part of, the H-2 complex in coding for TASA on tumors susceptible to immune surveillance (*12*).

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Antibacterial Synergism: A Proposal for Chemotherapeutic Potentiation Between Trimethoprim and Sulfamethoxazole

Abstract. *Sulfamethoxazole and other sulfa drugs are moderately potent inhibitors of* Escherichia coli *dihydrofolate reductase. They also significantly potentiate the inhibition of this enzyme by trimethoprim. The molecular basis for inhibition potentiation is the simultaneous binding of trimethoprim and sulfa by the enzyme. This potentiation may explain the synergism observed when these drugs are used in antibacterial chemotherapy.*

When used together in chemotherapy, sulfonamides and certain diaminopyrimidine derivatives exhibit mutual potentiating effects, or synergism (*1*). Their combined effectiveness far exceeds mere addition of their individual efficacy. One such combination (trimethoprim plus sulfamethoxazole) is in widespread current use (*2*). Diaminopyrimidines, such as trimethoprim and pyrimethamine, inhibit dihydrofolate reductase (E.C. 1.5.1.3) by competing with dihydrofolate (*3*). Sulfonamides, like sulfamethoxazole, are inhibitors of the enzyme dihydropteroate synthetase (E.C. 2.5.1.5) (*4*); they compete with the substrate *p*-aminobenzoate (*5*). Potter's (*6*) theory of "sequential inhibition" often is invoked (*2*, *7–9*) to explain the mutual potentiation between sulfonamides and diaminopyrimidines, but Webb (*10*) has pointed out that the sequential blockade of linear reactions by multiple inhibitors in the steady state is theoretically incapable of producing an effect greater than that by a single inhibitor alone, an effect confirmed experimentally by Rubin *et al.* (*11*). Furthermore, the theory of "sequential inhibition" offers no explanation for the fact that some dihydrofolate reductase inhibitors, such as 2,4-diaminopteroylaspartate, are not synergistic with sulfonamides (*9*), or that the potentiation of trimethoprim by sulfonamides still occurs in a number of sulfonamide-resistant organisms (*12*). Hitchings summarized (*13*) arguments for and against the theory, and concluded that "sequential inhibition" was not yet disproved.

Recent observations in this laboratory that sulfonamides can be moderately potent inhibitors of bacterial dihydrofolate reductase (*14*) provide the basis for a new theory of potentiation. This new hypothesis is based on multiple simultaneous inhibition of bacterial dihydrofolate reductase by the sulfonamides and their potentiators acting together. I present data to show that the qualitative nature of synergistic dose-response curves can be duplicated in vitro by the action of two inhibitors on a single enzyme. The