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Induced Melanoma Rejection[1]

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Treatments which heighten the immune response can inhibit tumor growth in susceptible hosts. For example, BCG administered with tumor cells or injected into established intradermal tumors (1, 2) resulted in specific, systemic tumor immunity in guinea pigs. These procedures, however, depended upon direct contact of BCG and tumor cells; contralateral challenges were less effective (3). Another approach, effective with cutaneous neoplasms, involved the use of sensitizing agents such as dinitrochlorobenzene (DNCB) or purified protein derivative (PPD) of tuberculin (4). Such agents elicited delayed hypersensitivity reactions (cellular immunity) at sites of neoplastic lesions, followed by regression and reduced incidence of new lesions. This form of local sensitization therapy, however, requires prior identification of superficial neoplastic lesions, and might not generate systemic tumor immunity if applied some distance away from tumor cells.

In mice, alloantigenic tumor cells stimulating a host rejection response have provided protection against other tumor cells to which the untreated hosts are susceptible (5, 6). In the latter study, alloantigenic (B16) melanoma implantation preceded by one week the contralateral challenge by Harding-Passey (H-P) melanoma to H-P-susceptible BALB/c mice, resulting in an induced systemic immunity against the H-P melanoma. But, not every H-P-challenged BALB/c recipient was adequately protected by the alloantigenic B16 melanoma pretreatment. Only 40% rejected their H-P implants, about 30% exhibited retarded H-P growth, and 30% indicated no anti-H-P protection when compared with untreated control hosts. We have, therefore, sought, and found, more effective protection in the form of strongly alloantigenic normal tissue implants, i.e., spleen and liver cells. Moreover, the use of more precisely genetically controlled antigenic variants found in various inbred mouse strains has led to results tending to rule out explanations of induced tumor rejection due to cross-reactions. Our findings are consistent with the possible adjuvant effect of alloantigenic tissue implants leading to augmentation of an otherwise inadequate tumor immunity. Finally, the results indicate that the induced tumor immunity is systematic and persistent.

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MATERIALS AND METHODS

Mice. Our most extensively used test system consisted of the highly susceptible recipient strain, BALB/c ($H\text{-}2^d$); the transplantable Harding–Passey melanoma; and strain C57BL/6 ($H\text{-}2^b$) donors of normal spleen and liver. A preliminary experiment involved the congenic strains A/J ($H\text{-}2^a$) and A.BY ($H\text{-}2^b$), serving, respectively, as normal tissue donor strain and as tissue and melanoma-recipient strain. These congenic strains have been bred so as to be identical for all histocompatibility factors, except for the H-2 difference.

Preimmunization. One donor spleen was minced in 0.2 ml of Tissue Culture Medium 199 (Difco), drawn into a syringe and implanted subcutaneously into a like-sexed H-P-susceptible recipient. The same donor's liver (without the gallbladder), providing three to four preimmunizing doses, was minced in 0.4 ml of Medium 199 before subcutaneous implantation into like-sexed recipients.

Tumor challenge. H-P melanoma minces were prepared without any added materials. Subcutaneous implants of 0.06 ml minced tumor resulted in practically 100% takes in unprotected control BALB/c hosts. When the hosts were pretreated with normal tissue implants, the melanoma challenges were administered contralaterally either on the same day or 1 week later. In one preliminary trial, contralateral tumor challenge followed allogeneic liver implantation by 1 month. Xenogeneic spleen and liver implants, obtained from deer mice (*Peromyscus maniculatus*), were also tested for antitumor protection. Finally, second and third tumor challenges were performed contralaterally to the previous tumor challenge sites.

Controls. Two types of controls were used: (1) untreated individuals, challenged by tumor only, and (2) syngeneic (i.e., BALB/c) spleen or liver implantation followed by contralateral tumor challenge. Whenever possible, littermates were assigned to both experimental and control treatment groups.

Treatment effects. Tumor size was estimated by the product of externally measured greatest and least diameters (in mm^2). Complete tumor rejection was concluded when no recurrence was noted 2–4 weeks after disappearance of all external signs of tumor presence. Tumor growth retardation was scored when the tumors of the pretreated experimental host animals exhibited at least a 2-week delay in achieving progressive growth when compared with the slowest growing tumors in the corresponding control groups.

Persistence of tumor immunity. The time interval between the disappearance of a previous tumor and the challenge by another tumor provided a measure of persistence of tumor immunity.

RESULTS

Antitumor protection of BALB/c hosts by C57BL/6 allografts is demonstrated in Table 1. The following points are noteworthy:

1. All (28) untreated controls succumbed to progressive tumor growth.
2. All (11) syngeneic (BALB/c) spleen or liver pretreated controls exhibited no antitumor protection.
3. All (6) allogeneic (C57BL/6) tissue pretreated hosts succumbed like untreated controls when tissue implants and tumor challenges were performed on the same day.

TABLE 1

ANTITUMOR PROTECTION BY ALLOGENEIC TRANSPLANTS
(*H*-2 and non-*H*-2 differences)

	Treatment	Like controls	Retarded	Rejected	Total different individuals
1	Untreated BALB/c controls (takes)	28/28	—	—	28
Δ t[a] = 1 week					
2	Syngeneic spleen (BALB/c)	6/6	—	—	6
3	Syngeneic liver (BALB/c)	5/5	—	—	5
4	Allogeneic spleen (C57BL/6)				
	1st H-P challenge	—	—	12/12	12
	2nd H-P challenge	—	4/12	8/12	
	3rd H-P challenge	—	—	5/5	
5	Allogeneic liver (C57BL/6)				
	1st H-P challenge	—	1/8	7/8	8
	2nd H-P challenge	—	1/7	6/7	
	3rd H-P challenge	—	—	2/2	
Δ t = 0 days					
6	Syngeneic spleen	2/2	—	—	8
7	Allogeneic spleen	1/1	—	—	
8	Allogeneic liver	5/5	—	—	
Δ t = 1 month					
9	Syngeneic liver	1/1	—	—	2
10	Allogeneic liver	—	1/1?	—	
Total					69

[a] Δ t = interval between normal tissue graft and contralateral tumor challenge.

4. A 1-week interval between allograft implantation and tumor challenge resulted in significant antitumor protection. All (12) spleen recipients rejected their first challenge tumors. When subsequently rechallenged, 8/12 hosts rejected a second time, while the 4/12 hosts succumbing to their second tumors exhibited tumor growth retardation. Five of the eight second-challenge survivors were then challenged a third time. All (5/5) rejected their third challenge tumors.

5. Comparable antitumor protection was provided by liver allografts. All but one (7/8) hosts rejected their first challenge tumors. However, the tumor growing progressively exhibited retarded growth. The seven survivors were rechallenged, and all but one (6/7) rejected their second challenge tumors. Again, the single tumor escaping the host defenses exhibited retarded growth. Two of the six second-challenge survivors were challenged yet a third time. Both rejected their third tumors.

6. In a preliminary test involving one untreated control, one syngeneic liver control, and one allogeneic liver experimental animal, with tumor challenges performed 1 month after pretreatments, the tumor in the allogeneic liver pretreated host grew more slowly than did the tumors in the other hosts. This preliminary result, which must be verified with greater numbers of tested individuals, suggests the possible persistence of some antitumor protection after allogeneic implantation.

Clearly these treatments have induced a strong and systemic antitumor immunity. This immunity is also persistent as indicated in Table 4. Allogenic spleen recipients challenged a second time as long as 81 days after first tumor disappear-

TABLE 2

ANTITUMOR PROTECTION BY ALLOGENEIC TRANSPLANTS
(*H-2* difference only)

Treatment	Like controls	Retarded	Rejected	Total different individuals
Untreated A.BY controls (takes)	5	—	2	7
Δ t = 1 week				
Allogeneic spleen (A/J)				
1st H-P challenge	—	—	2/2	2
2nd H-P challenge	—	—	2/2	
Allogeneic liver (A/J)				
1st H-P challenge	—	—	5/5	5
2nd H-P challenge	—	—	5/5	
Total				14

TABLE 3

ANTITUMOR PROTECTION BY XENOGENEIC TRANSPLANTS

Treatment	Like controls	Retarded	No take	Total different individuals
1 Untreated BALB/c controls (takes)	7/8	—	1/8	8
Δ t = 1 week				
2 Xenogeneic[a] spleen	—	2/2	—	2
3 Xenogeneic liver	3/6	2/6	1/6	6
Total				16

[a] Xenogeneic donor: *Peromyscus maniculatus* (deer mouse).

ance, and challenged a third time as long as 88 days after second tumor disappearance, were still capable of tumor rejection. Similar results were obtained with allogeneic liver. A second challenge tumor implanted 85 days after first tumor disappearance exhibited retarded growth. In the 6/7 second tumor rejections, recipients challenged as long as 102 days after first tumor rejection still exhibited strong tumor immunity. Finally, in the case of the third tumor rejections, strong tumor immunity persisted in one case for at least 46 days, and at least 73 days in the other.

Preliminary studies with another test system, involving the congenic A/J donor and A.BY recipient strains corroborate the results of the C57BL/6 donor and BALB/c recipient system (Table 2). The A.BY strain seems somewhat more resistant to Harding–Passey implants than BALB/c, at both the basal level of untreated controls and at the augmented level induced by allografts. While the C57BL/6 and BALB/c strains differ at both *H-2* and non-*H-2* gene loci, the congenic A/J and A.BY presumably differ only at the *H-2* locus. This single difference is sufficient to induce strong, systemic tumor immunity.

Antitumor protection provided by *Peromyscus* xenografts (Table 3) appears to be decidedly weaker than allograft protection. Preliminary tests still in progress already indicate that half (3/6) of the tumors in xenogeneic liver recipients (BALB/c hosts) grow as quickly as the tumors in untreated control hosts.

TABLE 4
PERSISTENCE OF ANTITUMOR IMMUNITY

Treatment	Δt_1 (days)[a]		Δt_2 (days)[b]	
	Range	*Mean*	*Range*	*Mean*
Allogeneic spleen				
Retarded takes (4/12)	7–81	44.3	—	—
Rejections (8/12)	7–81	24.5	—	—
Rejections (5/5)	—	—	78–88	83.2
Allogeneic liver				
Retarded take (1/7)	—	85	—	—
Rejections (6/7)	20–102	45.7	—	—
Rejections (2/2)	—	—	46,73	59.5

[a] Δt_1 = Interval between no sign of first tumor and second tumor challenge.
[b] Δt_2 = Interval between no sign of second tumor and third tumor challenge.

DISCUSSION

Our current and previous results (6) suggest four levels of induced antitumor immunity in the following descending order of pretreatment effects: (1) allograft, (2) allogeneic tumor, (3) xenograft, and (4) syngeneic tissue, equivalent to untreated control. The reasons for these different levels of augmented tumor immunity are not clear. Nevertheless, our testing procedure has provided a sufficiently sensitive *in vivo* assay system to distinguish among several different levels of effect with respect to induced antitumor immunity.

Our double tumor challenge system (6) was consistent with the hypothesis that induced rejection of Harding–Passey melanoma by BALB/c hosts resulting from allogeneic B16 melanoma implantation might be due to host cross-reaction to weak tumor-associated cell surface antigens in both B16 and H-P tumors. Such an explantation could not be invoked to account for protection by normal allografts. However, in the C57BL/6–BALB/c donor–recipient system it was still possible to postulate that the C57BL/6 allografts provoke strong cross-reactive responses to weak antigens common to both C57BL/6 spleen or liver and Harding-Passey melanoma, but not present in the BALB/c host strain. Such an explanation cannot hold in the case of the congenic A/J–A.BY donor–recipient system, since these strains presumably differ only at the *H-2* locus. Therefore, they cannot differ by weak non-*H-2* antigens, and hence provide no basis for cross-reactive tumor rejection. The simplest explanation of our results to date is that the incompatible grafts constitute immunogenic stimuli which simply augment the level of cell-mediated antitumor immunity directed against tumor-specific antigens. Thus, this form of immunologic intervention assists the host by augmenting the level of antitumor activity from an ineffective level to an effective one.

It may be argued that normal allogeneic spleen transplants provide adoptive antitumor immunity rather than augment the host's own antitumor activity. In reply it might first be noted that liver allografts are about as effective as normal spleen allografts, although it might be expected that spleen should be more effective due to a greater number of presumptive "killer" cells. Second, both spleen and liver allografts, if they provide adoptive immunity, should exert maximum tumor-rejecting activity when implanted with tumor on the same day, before these allografts are themselves rejected by the host. Just the reverse is observed; no antitumor effect when allografts and tumor challenges occur on the same day, but rather an

optimal effect when tumor challenge follows allograft by a week, by which time allogeneic "killer" cells might themselves be rejected by an immunologically competent host.

Finally, we have not yet observed gross indications of severe stress associated with allogeneic implants and successive tumor challenges. It is now 10 months since the five spleen-pretreated, three-time tumor rejecting hosts received their allografts, and they are still alive and apparently well. In this case the cure is better than the disease.

SUMMARY

1. Strong, systemic and persistent antitumor immunity has been induced in susceptible individuals by administering strongly alloantigenic normal tissue, spleen or liver, prior to contralateral tumor challenge.

2. Two inbred mouse donor–recipient combinations were used: C57BL/6 allografts into BALB/c recipients, and A/J donors with congenic A.BY recipients. Both recipient strains are highly susceptible to the Harding–Passey (H-P) melanoma.

3. Allograft recipients, when challenged 1 week later by contralateral implantation of H-P melanoma nearly always rejected their first tumor challenges.

4. Persistence of antitumor immunity was indicated by rejections of second tumor challenges made as long as 102 days after previous tumor disappearance, and by rejections of third challenge tumors implanted as long as 88 days after previous tumor disappearance.

5. Xenografts from the deer mouse, *Peromyscus maniculatus,* resulted in augmented tumor immunity, but such grafts were not as effective as allografts.

6. Syngeneic tissue implants, or allografts performed on the same day as the tumor challenge provided no significant antitumor protection.

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