Lymphoid Index in Allogeneic Inhibition[1] (36386)

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It is well established that growth of a parental line tumor is inhibited in the F_1 hybrid mouse. This phenomenon has been designated as allogeneic inhibition or hybrid effect. Investigations have led to divergent views regarding its mechanism. Some have related this inhibition to the immunologic vigor exhibited by hybrids (1–3), whereas others consider the reaction to be nonimmunologic (4–6). The most compelling evidence in support of the latter contention has been studies which reveal comparable *in vitro* effects of agents which abrogate this phenomenon *in vivo*. On the other hand, thymectomy supplemented by irradiation which suppresses the immune response or the administration of a nonspecific immunologic stimulant as killed *Mycobacterium tuberculosis* recently have been noted to respectively decrease or increase the resistance to tumor growth in the F_1 hybrid (3).

We have recently described a relatively simple technic for evaluating the immunologic response of rats to tumor growth (7). A distinct band of lymphoid cells appeared at the interface of tumor and kidney following implantation of a methylcholanthrene (MC) tumor beneath the renal capsule in Lewis rats with sinecomitant and concomitant immunity (8). This response was either absent or negligible in nonimmune syngeneic rats receiving similar tumor inoculation. Because of this it was considered worthwhile to evaluate the lymphoid response at the interface of C3H mammary tumors and kidney in F_1 hybrids.

Materials and Methods. The first experiment was performed to determine the validity and usefulness of estimates of the lymphoid response at the interface of kidney and tumor in mice as had been demonstrated previously in the rat. Uniform plugs of spontaneous C3H mammary tumors in their 15–20 generation of transplantation were placed beneath the renal capsule of female syngeneic C3H recipients that were either nonimmune or treated so that they exhibited sinecomitant or concomitant immunity as described previously (8). The immune status was verified by noting tumor growth 35 days after subcutaneous challenge doses of 1×10^4 C3H mammary tumor cells in the ankle of immune and nonimmune recipients.

In the second experiment uniform plugs of C3H mammary tumors were similarly placed beneath the renal capsule of C3H, DBA/2J female mice and their F_1 offspring, the $C3D2F_1/J$ strain. The hybrid effect was verified by subcutaneously inoculating 15 mice of these lines in the ankle with 1×10^4 mammary tumor cells and estimating tumor growth 35 days later.

Groups of animals from each experiment were sacrificed 5, 7, 10 and 14 days after subcapsular renal implantation. Their kidneys were transected so that blocks contained the tumor–kidney interface. These were fixed in Zenker's acetic fluid, dehydrated and imbedded in paraffin in the usual manner. Sections were stained with hematoxylin, eosin and methylene blue. The number of lymphoid cells encountered at the tumor kidney interface was subjectively quantitated (0–3+) in each animal. The total score for members of a group were divided by the number of samples yielding a lymphoid index. The significance of differences between groups was estimated by the Student's *t* test.

Results. Only an occasional nonimmune

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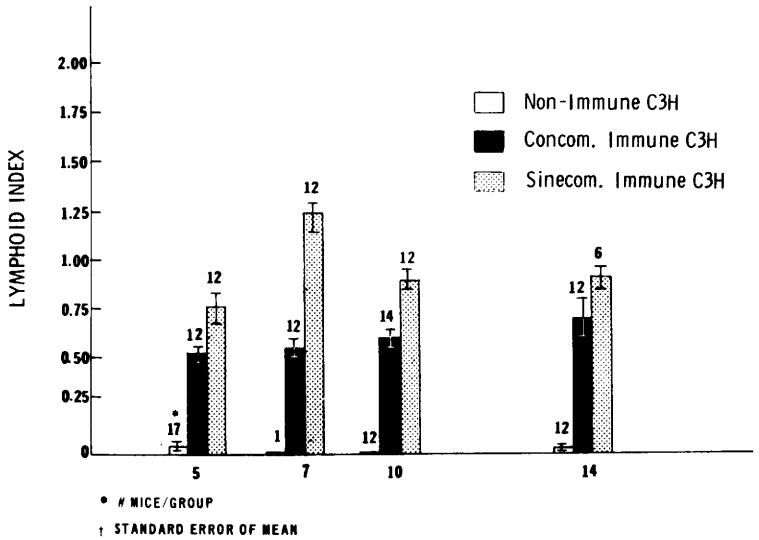

FIG. 1. Lymphoid index in nonimmune and sinecomitant and concomitant immune C3H mice 5, 7, 10 and 14 days after subcapsular renal implants of C3H mammary tumor.

C3H mouse exhibited a slight lymphoid response at the tumor–kidney interface at 5 and 14 days after implantation of a plug of C3H mammary tumor beneath the renal capsule (Figs. 1 and 2). The response was significantly ($p < .01$) greater in animals with sinecomitant and concomitant immunity than in nonimmune members and was greater ($p < .01$) in those with the former than the latter type of immunity (Fig. 1).

A slight lymphoid response was also observed in C3HD2F$_1$/J mice which was not statistically different ($p > .05$) from that observed in the parent C3H mice but was significantly less ($p < .01$) than that observed in the DBA parental line (Fig. 2).

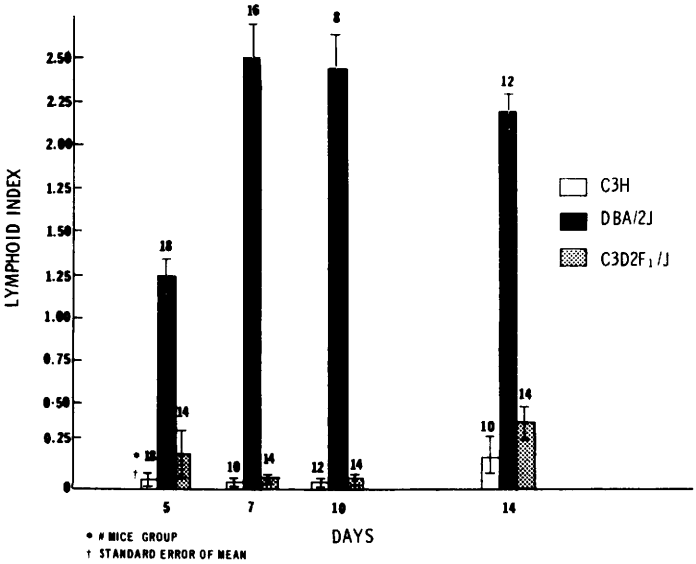

FIG. 2. Lymphoid index in C3H, C3D2F$_1$/J and DBA/2J mice 5, 7, 10 and 14 days after subcapsular renal implants of C3H mammary tumor.

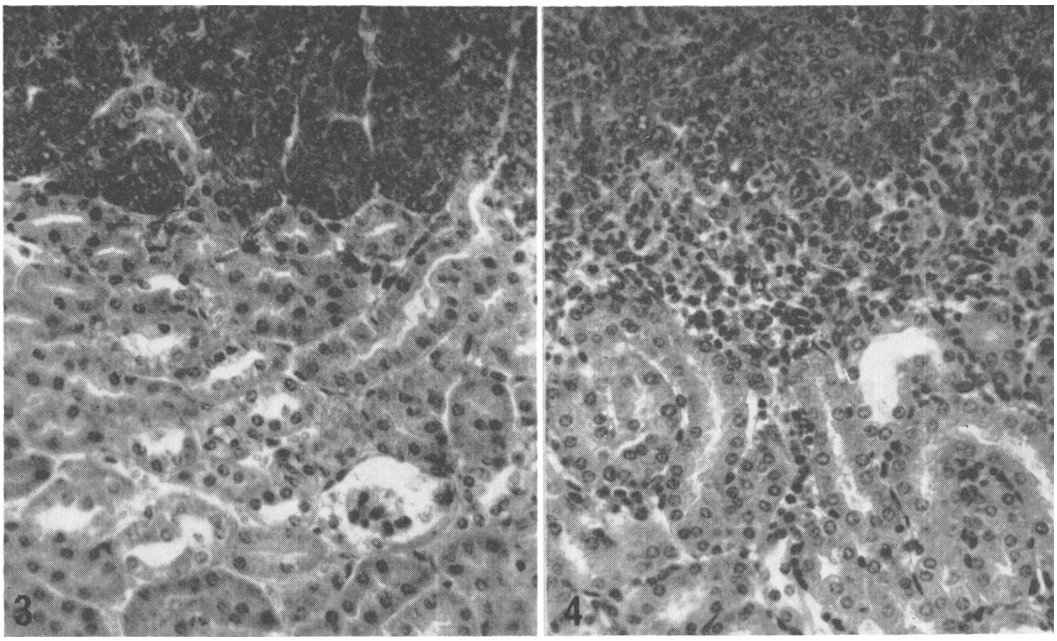

FIG. 3. Tumor kidney interface as it appeared in most nonimmune and F_1 hybrid mice. No reaction is present (×250).

FIG. 4. A moderate (2+) lymphoid reaction is present at the tumor kidney interface. This appearance was encountered in most immune syngeneic and allogeneic DBA mice (×250).

The lymphoid response was comprised principally of adult and lesser numbers of immature lymphocytes (Figs. 3 and 4). A few plasma cells were noted in the infiltrate at 14 days after tumor inoculation.

Although actual measurements of the tumors were not made the distinct impression was gained that tumors were smaller at the 7, 10 and 14 day periods in immune and F_1 animals than in those that were nonimmune. No consistent differences in the histologic appearance of the tumors was encountered at any time period in both experiments performed except that tumor necrosis was observed in most DBA and some F_1 hosts at 5 and 7 day intervals. Moderate fibrosis was encountered in tumors in mice of these groups at 10 and 14 days.

Only 33% of mice with sinecomitant and concomitant immunity that received subcutaneous inocula of tumor exhibited growth at 35 days. Thirteen percent of hybrids and no DBA mice had tumor growth at this period although tumors were noted in 90% of C3H recipients (Table I).

Discussion. These results confirm our previous observations which revealed the association of a lymphoid response at the interface of MC tumors and kidneys of syngeneic Lewis rats with concomitant and sinecomitant immunity (7). As in that study it was noted that a few nonimmune animals exhibited a slight lymphoid response. The true significance of this reaction is uncertain. It is possible that it may represent a concomitant immune response to the tumor inoculum placed beneath the renal capsule. Nevertheless, the paucity of lymphoid elements in these rare instances may be readily distinguished from the more consistent and intense

TABLE I. Tumor Growth Following Subcutaneous Inoculation of C3H Mammary Tumor.

	No.	% Tumor
C3H nonimmune	30	90
C3H sinecom. immune	15	33
C3H concom. immune	15	33
$C3D2F_1/J$	15	13
DBA/25	15	0

reactions observed in the immune animals. Mice in this study, as was noted previously with rats which were immunized by subcutaneous implants of the same tumor line utilized for subcapsular renal implantation, exhibited a significant resistance to subsequent challenge doses of tumor cells. This information warrants our conclusion that this lymphoid index, in accord with prevalent views of the cellular nature of tumor immunity, represents a valid and useful indicator of such reactivity in the systems explored. Identical results have also been recently noted in our laboratory with an MC tumor induced in C3H mice.

In this light we have interpreted the sparse, insignificant lymphoid response at the C3H mammary tumor kidney interface of F_1 hybrid mice as evidence against the view which regards allogeneic inhibition or the so-called hybrid effect as an immunologic phenomenon. Again, it is noteworthy that a large percentage of F_1 hybrids resisted subcutaneous inocula of the same C3H tumor in the ankle.

This interpretation is divergent from that recently presented by Sanford and Soo (3). Although these investigators observed a moderate increase in tumor growth in F_1 hybrids subjected to thymectomy and irradiation, the confirmation of the immunologic status of their animals was not presented. They also noted increased resistance to tumor growth in F_1 hybrids following treatment with the nonspecific immunologic stimulant *M. tuberculosis*. It appears germane to note that we have observed abrogation of F_1 resistance in the same tumor system employed in these studies following administration of antilymphocyte serum (ALS). However, incubation of tumor cells in small, systemically ineffectual amounts of ALS *in vitro* also abrogated the resistance of these F_1 hybrids indicating that the effect of ALS in this regard was nonimmunologic (6).

Summary. A distinct band of lymphocytes was observed at the interface of subcapsular renal implants of C3H mammary tumors and kidneys of syngeneic C3H mice with sinecomitant and concomitant immunity and the allogeneic DBA/2J strain. This reaction was either absent or significantly less in nonimmunized C3H mice and the $C3D2F_1/J$ hybrid. This information is interpreted as evidence against the immunologic nature of so-called allogeneic inhibition or hybrid effect as it relates to tumor growth.

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