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A Study of the Functional Capacities of Spleen Cells After Maintenance *in vitro*.* (31403)

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The ability of cells to retain their unique antigenic character while being held in tissue culture is a well documented fact. Mannick *et al*(1), have shown that rabbit spleen cells may be cultured for as long as 6 weeks and still elicit a second set reaction when used as a first set homograft. That is, the histocompatability antigens remained intact for at least this period. Tumor tissue has also been shown to retain its unique antigenic characteristics for very long periods both *in vitro* and when serially transferred in animals (2).

On the other hand, it has also been demonstrated that severe structural(3) and functional changes can occur in organized tissues that are held in culture for even short periods of time. In our laboratory, it also has been shown that the sensitivity of the structural changes observed in cultured tissue are a function of the complexity of the medium(4) and/or additives such as adrenal corticoids (5).

Little information is available concerning the changes in the functional status of lymphoid tissues *in vitro*. Michaelides and Coons(6) have shown that sensitized lymphocytes are capable of secondary production of antibody for a limited time (4 to 8 days) *in vitro* depending upon the type of antigens added to the culture medium. In addition, Toa and Uhr(7) have shown that primary sensitization of lymphocytes is possible in culture indicating that this aspect of cellular function may be retained for some time *in vitro*. The transformation *in vitro* of peripheral blood lymphocytes to large polyblastic cells in response to the presence of allogenic cells or phytohemagglutinin(8) probably also represents the retention of some degree of functional capacity in culture for at least short periods. However, none of the work cited contains any information concerning the overall functional status of the tissues during and after culture. That is, it is impossible to determine if the lymphatic cells are capable of returning to their original *in vivo* environment and function as a unified cellular system capable of protecting the animal against antigenic insult. Otherwise, the cell population must be viewed as a loosely integrated system as is seen in cell culture capable, at best, of very limited fuction. As a means of measuring the effect of the structural

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changes that occur in lymphoid tissues held in culture for varying lengths of time, we have used the ability of such cells to protect an animal against the late effects of lethal doses of irradiation. Survival of the lethally irradiated animal injected with cultured lymphoid tissue would seem to represent a test of the functional capacity of at least the necessary segments of the cultured cellular population.

Materials and methods. Strain A/jax female mice weighing between 15-25 g were given 900 R of whole body irradiation. The animals were housed in individual compartments in a lucite cage during irradiation. Immediately following irradiation, the mice were randomly divided into 3 groups. One group remained untreated and served as a control of the irradiation damage. A second was given fresh spleen cells prepared as follows. Fresh spleens were removed asceptically, minced into 5 mm^3 fragments. The cells were then gently expressed through a strainer into unsupplemented Eagle's Medium. The spleen cells were centrifuged and resuspended in approximately 0.25 cc of Eagle's Medium. A suspension of 5-10 $\times$ 10^6 viable cells was then injected into the tail vein of the irradiated animals. Two-month survival of the lethally irradiated animals was considered successful protection by the spleen cells. The third group of lethally irradiated animals received tissue cultured spleen cells prepared as follows. The spleens were cut into small fragments measuring 2 mm^3 and introduced into D 5.0 Carrell flasks with 5 cc of Eagle's Medium supplemented with 8% fetal calf serum. The cultures were incubated at 37°C for varying lengths of time. The medium was freshened every 2 days by careful removal of 2 cc of exhausted medium and replacement with 2 cc of fresh medium. At the end of the culture period, the flasks were agitated to loosen cells adherent to the floor of the flask. The cells with the medium were pipetted into centrifuge tubes and spun down. The cells were then resuspended in 0.25 cc of unsupplemented Eagle's Medium. A sample was removed and a viable cell count was taken by the erythrosin B exclusion technic (9). Any sample showing a viable cell count of less than 5 $\times$ 10^6 viable nucleated cells was eliminated. A suspension of 5-10 $\times$ 10^6 cultured cells was then injected into the tail vein of the lethally irradiated mice. Protection was again judged by a 2-month post-irradiation survival of the host animal.

TABLE I. Survival of Lethally Irradiated Animals Injected with Cultured Spleen Cells.

Days *in vitro*	Animal survival	%
0 (fresh spleen)	24/24	100
3	10/12	84
5	9/12	75
7	3/12	25
9	0/12	0
Uninjected controls	0/24	0

Results. The results are summarized in Table I. As can be seen, there was a relatively early loss (9 days) in the ability of cultured cells to protect lethally irradiated mice against late mortality. There appears to be a relatively stable period during the first 5 days *in vitro*. After 5 days, however, there is a rapid decline in the ability of spleen cells to protect the animals against late irradiation death.

None of the control, untreated, lethally irradiated animals survived. This assures that survival of the animals must be explained in terms of the ability of the cultured spleen cells to carry out successfully their original *in vivo* function. Enough cytological organization was retained so that the cells were able to function as a loosely organized system capable of mounting some sort of immunological response when challenged.

Despite the extended culturing period and the loss of some cells during replacement of the medium, there was a slight increase in the viable cell count probably due to mitotic activity of some elements of the cell population. Total cell counts per animal dose also remained in excess of the number needed to protect the animal if fresh, uncultured cells were given.

Histologic analysis of the cell population in the culture flasks indicates at least some transformation of the spleen cells to other cell types. Long term cultures, as expected, indicate a tendency toward the formation of giant and epithelial cell types.

Discussion. It is apparent from these re-

sults that the functional capacities of cultured lymphoid tissues are very rapidly dissipated *in vitro* under the culturing conditions used here. The rapid loss of the loose organization of splenic tissue is surprising in view of other results obtained using skin as organ culture material. We have shown(5) that skin may be cultured in a comparable system for at least 10 days with a 70% chance of returning successfully as an autograft to the original *in vivo* environment. Basic differences in metabolic rates and levels of differentiation may explain these results.

The likeliest interpretation of our results appears to be transformation of the splenic cells *in vitro*. The release of the lymphoid cells from *in vivo* control results in the transformation of the spleen cells into other cell types that either are incapable of reorganization into immunologically sensitive systems or have lost at least a part of their cyto-immunologic makeup but that this aspect is essential to totally protect the lethally irradiated animal. The fact that cultured lymphocytes have been shown to be capable of primary antibody response(7) seems to argue for the latter interpretation.

Transformation of the lymphoid cells *in vitro* is routinely observed in cell cultures of lymphoid tissue. Recently, Sutton and Weiss (10) have elegantly described the subcellular cytological changes that occur in monocytes when they are transformed in culture. These authors state that transformation begins on the third to fourth day *in vitro* and usually ends by 5 to 6 days. Our results correlate quite well with their data since the loss of function in our system became most evident between 4 to 6 days and was nearly complete by the seventh day. That is the time when a critical number of cells have been transformed. The organized quality of the spleen cell organ culture was lost and the system became transformed into a cell culture incapable of protecting the host against irradiation.

In addition, it is probable that the transformation of the spleen cells was irreversible since there was no pathological evidence to indicate that the death of the animals receiving the 9-day cultured cells was any different from that of the animals receiving no treatment after irradiation. However the possibility that reversal of the transformation seen *in vitro* may require too long a time period *in vivo* to influence the survival of the lethally irradiated animal cannot be ruled out by these data.

Finally, from these data, it is evident that great caution must be exercised in interpretation of results obtained using cultured lymphoid tissues. Choice of the kind of medium and culture system used is of great importance. Long term results obtained using systems similar to those reported herein should be viewed with caution and with the idea in mind that severe structural and functional changes can and do occur. It is apparent that further research in this area is needed. The addition of compounds such as steroids that are known to suppress *in vitro* cytological alterations should be investigated for their effects upon cultured lymphoid tissues.

Summary. Spleen cells were cultured for varying lengths of time *in vitro*. The cells were then injected into lethally irradiated mice to ascertain their ability to protect the animals against irradiation death. Complete loss of ability to protect irradiated animals was found to occur after 9 days *in vitro* despite the injection of adequate numbers of viable spleen cells. The significance of these results is discussed.

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