Reprinted from PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE
1968, v128, 952-954

# Immunoglobulin Production by Embryonic Tissues: Thymus Independent (33164)

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Previous reports (1,2) have shown that cells capable of producing immunoglobulins are present in the liver of the mouse embryo by the fourteenth day of gestation. When transferred to lethally irradiated hosts, these lymphoid precursors, while dependent upon intact thymic function for their ability to produce detectable specific antibody in response to antigenic stimulation were able to synthesize immunoglobulins in the absence of the thymus. These findings suggested that while the thymus may promote the ability of these lymphoid precursors to form specific antibody, immunoglobulin synthesis *per se* is independent of the thymus. The initial experiments, however, did not rule out the possibility that the transferred cells may in fact have been post-thymic cells with previously determined antigenic commitment. That is, the immunoglobulins produced by these cells could have been antibodies to antigens contacted shortly after transfer. In this report it will be shown that cells which have the potential to produce immunoglobulins in thymectomized irradiated hosts are present in the yolk sac, placenta, and liver of the mouse embryo prior to the appearance of the thymus.

*Materials and Methods.* Pregnancies were surgically interrupted at various stages of gestation. The embryos and extra-embryonic tissues were dissected free of maternal tissue; they then were washed twice in cold TC 199 (Difco) in an effort to eliminate the possibility of contamination of the extra-embryonic tissues by maternal blood. Identical organs or tissues from a single pregnancy (5–9 embryos) were pooled in cold TC 199 and the cells were gently dissociated by mincing and aspiration through a 20-gauge needle. The age of the gestation was based on the following criteria: 7–8 days, a well developed ectoplacental cone and early membrane formation; 9 days, a well-developed yolk sac, a conspicuous heart, and no liver pigment; 10 days, early pigmentation in the liver; 11–12 days, a well-pigmented liver and the absence of the thymus. One host was the recipient of the pooled identical organ from a single pregnancy. The cell suspensions were injected intraperitoneally into the irradiated hosts. Tissues from C3H.SW/Sn embryos were transferred to thymectomized adult congenic hosts (CWB/5Herz) (3) which had received 500 rad whole-body X-radiation; tissues from CBA/J embryos were transferred to thymectomized (C57Bl/6 $\times$ DBA/2) $F_1$ mice which had received 870 rad X-radiation. Absence of the thymus was confirmed at autopsy. The above strain com-

TABLE I. Production of Gamma-2a Globulins by Various Fetal Tissues when Transferred to Thymectomized, Lethally Irradiated Allogeneic or Sublethally Irradiated Congenic Mice.[a]

| Fetal tissue given | Age of embryo (days) | | | |
|---|---|---|---|---|
| | 7–8 | 9 | 10 | 11–12 |
| Liver | | | 3/3 | 4/4 |
| Gut | | | | 0/3 |
| Lung | | | | 0/3 |
| Femur | | | | 0/2 |
| Skin | | | | 0/2 |
| Yolk sac | 0/1 | 3/3 | | 2/2 |
| Placenta | 0/2 | | | 1/1 |
| Above diaphragm | 0/2 | 0/1 | | |
| Below diaphragm | 0/2 | 3/3 | | |
| None | | 0/1 | 0/1 | 0/7 |

[a] The allogeneic hosts were bled 45 days and the congenic hosts 60 days after irradiation. The number of sera containing gamma-2a globulins of donor allotype are reported (no./total).

binations were chosen because they have readily distinguishable γG2a immunoglobulin allotypes (4). The lethally irradiated allogeneic hosts were bled from the retro-orbital plexus 45 days after irradiation, and the congenic hosts were bled 60 days after irradiation. The sera were assayed for donor γG2a globulins by an inhibition of precipitation assay (5).

In addition, tissues were taken from CBA/J mice as noted above and injected intraperitoneally into lethally X-irradiated (840 rad) or sublethally irradiated (600 rad) CBA-T6T6 mice. Approximately 30 days later these mice were killed and their tissues (thymus, spleen, and bone marrow) were prepared for chromosome analysis (only the results for the host thymus are reported here). In most instances, at least 25 metaphase plates were scored for each tissue. The finding of donor cells in the thymus of the host was interpreted as indicating the presence of lymphoid stem cells in the inoculum (6,7).

*Results and Discussion.* As can be seen in Table I, γG2a globulins of donor allotype were found in the sera of thymectomized hosts which had received yolk sac or the caudal half of 9-day embryos. Lymphoid precursors capable of immunoglobulin production were present in liver of 10-day embryos and in liver, yolk sac, and placenta of 11- and 12-day embryos. The results of the morphologic assay correlated very well with these findings (Table II).

It is apparent from these results that cells which have the potential to differentiate into immunoglobulin producing cell lines are present in the embryo and yolk sac by the ninth day of gestation. The thymus does not appear until at least 3 days later. Thus, while the thymus would appear to be essential for the production by these cells of detectable specific antibody in response to a least certain antigens, these results demonstrate that the thymus is not essential for immunoglobulin synthesis per se. Moreover, if *all* immunoglobulins are preprogrammed products of individual lymphoid cell clones, then the donor γG2a globulins found in these experiments may in fact have been antibodies in the general sense that they could combine with certain substances but not with others. That is, in the absence of the thymus, the immunoglobulins produced in response to an antigen may be of too low affinity to be serologically

TABLE II. Percentage of Donor Cells in the Thymus of Lethally or Sublethally X-Irradiated CBA-T6T6 Mice 30 Days After the Intraperitoneal Injection of Various CBA Embryonic Tissues.

| Fetal tissue given | Age of embryo (days) | | | |
|---|---|---|---|---|
| | 7–8 | 9 | 10 | 11–12 |
| Liver | | | 92, 100 | 100, 96 |
| Gut | | | 0, 0 | 0 |
| Lung | | | | 0, 0 |
| Femur | | | 0 | 0 |
| Skin | | | 0 | 0 |
| Yolk sac | 0, 0 | 95 | 84, 80 | 100, 33 |
| Placenta | 0, 0 | 90 | 90, 25 | 100, 0 |
| Above diaphragm | 0 | 0, 0 | 70, 100 | 96 |
| Below diaphragm | 0 | 90, 100 | 100, 60 | |
| None | | 0 | 0 | 0 |

detectable. Thus the role of the thymus may be to promote or to catalyze the increase in antibody affinity which occurs after immunization (8).

1. Tyan, M. L., Cole, L. J., and Herzenberg, L. A., Proc. Soc. Exptl. Biol. Med. **124**, 1161 (1967).

2. Tyan, M. L., Cole, L. J., and Herzenberg, L. A., in "Proceedings of the First International Congress of the Transplantation Society," Dausset, J., ed., Munksgaard, Copenhagen (1968).

3. Klein, J. and Herzenberg, L. A., Transplantation (1968) in press.

4. Herzenberg, L. A., Warner, N. L., and Herzenberg, L. A., J. Exptl. Med. **121**, 415 (1965).

5. Herzenberg, L. A., in "Regulation of the Antibody Response," Cinader, B., ed., Thomas, Springfield, Illinois (1967).

6. Ford, C. E. and Micklem, H. S., Lancet **6**, 359 (1963).

7. Tyan, M. L., Colē, L. J., and Nowell, P. C., Transplantation **4**, 79 (1966).

8. Eisen, H. N. and Siskind, G. W., Biochemistry **3**, 996 (1964).

Received March 14, 1968. P.S.E.B.M., 1968, Vol. 128.