PRODUCTION OF TL ANTIBODY BY MICE IMMUNIZED WITH TL- CELL POPULATIONS

A POSSIBLE ASSAY FOR THYMIC HORMONE*

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The TL (thymus leukemia) antigens are demonstrable only on thymocytes and leukemia cells (1, 2). Therefore to preclude the production of TL antibody, when immunizing mice against some other antigen, we have sometimes elected to immunize with spleen and lymph node cells, omitting thymocytes. Contrary to expectation, this does not necessarily prevent the formation of TL antibody, and so we were obliged to test which of the two following explanations might account for this paradox:

(a) TL antigen is present on cells outside the thymus (although such cells have never been demonstrated), and it is these that induce production of TL antibody on inoculation into TL- recipients.

(b) TL antigen is not present on cells outside the thymus, but precursor cells (which are known to be present in spleen and bone marrow and to repopulate the thymus of lethally irradiated mice with TL+ cells [3]) become TL+ after inoculation, under the influence of the recipient’s thymus, and then induce the formation of TL antibody.

The first experiment is summarized in Table I: Mice of the congenic A/TL- strain (4) were thymectomized between 4 and 6 wk of age, while others were subjected to the same operation without removal of the thymus (sham thymectomy). Approximately 1 wk later, mice of both groups received a subcutaneous inoculation of 2-3 X 106 washed cells (thymus, spleen, lymph node, or bone marrow) from A strain (TL+) donors. Starting 2 wk later, the immunization was repeated intraperitoneally five times at weekly intervals. 10 days after the last immunization the serum of each mouse was titrated against A strain thymocytes for the presence of TL antibody.

Among the sham-thymectomized mice, TL antibody was made by 8/8 mice immunized with spleen cells, 2/5 mice immunized with lymph node cells, and 3/5 mice immunized with bone marrow cells. Of the thymectomized mice, 5/5 immunized with thymocytes produced TL antibody, but only 1/18 mice immunized with spleen cells, 2/5 mice immunized with lymph node cells, and 3/5 mice immunized with bone marrow cells.

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munized with spleen cells, lymph node cells, or bone marrow cells [this one exception being a thymectomized mouse immunized with spleen cells, which gave the minimum titer tested for (1:10)].

This result clearly favored the second explanation given above, namely that TL antibody production is caused by maturation of TL- precursor cells in the inoculum into TL+ thymocytes, under the influence of the recipient's thymus, the exceptional mouse being either inadequately thymectomized or having aberrant thymic tissue (5).

### TABLE I

**TL Antibody Production by Thymectomized and Sham-Thymectomized A/TL- Mice Immunized with A Strain Cells**

| Treatment of recipient | Immunized with A strain cells from Thymus | Cytotoxic titer* of serum vs. A strain thymocytes (anti-TL) (reciprocal) |
|------------------------|------------------------------------------|------------------------------------------------------------------------|
| Thymectomy             | Thymus                                    | 640, 640, 320, 320, 320                                                |
|                        | Spleen                                    | 10, <10, <10, <10, <10, <10, <10, <10, <10, <10, <10, <10, <10, <10   |
|                        | Lymph nodes                               | <10, <10, <10, <10, <10, <10, <10                                       |
|                        | Bone marrow                               | <10, <10, <10, <10, <10, <10                                           |
| Sham thymectomy        | Spleen                                    | 320, 160, 160, 80, 80, 40, 40                                           |
|                        | Lymph nodes                               | 80, 40, <10, <10, <10, <10                                            |
|                        | Bone marrow                               | 80, 40, 40, <10, <10                                                   |

Interpretation: Intact mice can make TL antibody when immunized with cells from hemopoietic organs other than thymus, but thymectomized mice cannot.

* For each mouse individually (end point 50% cells lysed). Specificity control: all sera were negative (<10% cells lysed) against syngeneic (A/TL-) thymocytes.

† <10: serum negative (<10% cells lysed) at lowest dilution tested (1:10).

The second experiment was designed to test the possibility that the failure of thymectomized mice to produce TL antibody when immunized with hemopoietic cells other than thymocytes might be due to immunosuppression caused by thymectomy (even though thymectomy had been carried out long after birth); otherwise it might be argued that a thymectomized mouse is capable of responding only to cells containing a large amount of TL antigen, i.e., to thymocytes. For the second experiment, thymectomized and sham-thymectomized A/TL- congenic mice were used as before, but the immunizing cells were taken from another A strain congenic stock, A/Thy-1.1 formerly called A/θ-AKR (see reference 7). In this instance the recipient has the opportunity to respond not only to TL antigens but also to the antigen Thy-1.1
(formerly θ-AKR) (Table II). The immunizing schedule was as before, but the sera were tested against AKR thymocytes for anti-Thy-1.1 as well as against strain A thymocytes for anti-TL.

The result (summarized in Table III) was decisive. Immunization with either thymocytes or spleen cells produced roughly equivalent titers of anti-Thy-1.1

### Table II

**Phenotypes of the Four Mouse Strains Used**

| Strain     | Thy-1 | TL  |
|------------|-------|-----|
| A          | 2     | +   |
| A/TL⁻⁻⁻⁻⁻⁻ | 2     | -   |
| A/Thy-1.1  | 1     | +   |
| AKR        | 1     | -   |

### Table III

**Production of TL and Thy-1.1 Antibody by Thymectomized and Sham-Thymectomized A/TL⁻⁻⁻⁻⁻⁻ Mice Immunized with A/Thy-1.1 Cells**

| Treatment of recipient | Immunized with A/Thy-1.1 cells from | Cytotoxic titer* (reciprocal) vs. thymocytes from | |
|------------------------|--------------------------------------|--------------------------------------------------|---|
|                        | Strain A (anti-TL)                   | Strain AKR (anti-Thy-1.1)                        | |
| Thymectomv             | Thymus                               | 1280, 640, 640, 640, 640                          | |
|                        | Spleen                               | <10, <10, <10, <10, <10                           | |
|                        |                                      | 320, 320, 320, 320, 320, 100, 100, 100, 100, 80     | |
|                        |                                      | 1280, 1280, 320                                   | |
|                        | Sham thymectomy                      | Thymus                                           | |
|                        | Spleen                               | 320, 320, 160, 160                                | |
|                        |                                      | 640, 640, 320, 320                                | |

Interpretation: Absence of thymus in the recipient makes no difference to its capacity to make Thy-1 antibody, but precludes the formation of TL antibody except where the inoculum is TL⁺⁺⁺⁺, i.e., contains mature thymocytes.

* For each mouse individually. Specificity control: all sera were negative against syngeneic (A/TL⁻⁻⁻⁻⁻⁻) thymocytes.

in both thymectomized and sham-thymectomized groups. Immunization with thymocytes also produced roughly equivalent titers of TL antibody in both thymectomized and sham-thymectomized groups. But immunization with spleen, while producing TL titers of 160–320 in sham-thymectomized recipients, failed to produced any anti-TL response in thymectomized recipients. Immunosuppression is thus effectively ruled out as a cause for the disparate TL titers of thymectomized vs. sham-thymectomized mice immunized with spleen cells.

**Comment**

These findings show that the production of TL antibody by mice immunized with hemopoietic cell populations other than thymocytes depends on the
presence of a thymus in the recipient, and this implies that production of TL antibody by such mice requires first the maturation of TL- precursors into TL+ thymocytes. Whether the cells must go to the thymus for this to happen, or whether it can happen at the site of inoculation (subcutaneous or intraperitoneal) under the influence of a thymic hormone, is not decided by the present experiments. Possibly the capacity of the thymectomized TL- mouse to make TL antibody when immunized with spleen cells could form the basis of an assay for thymic hormone.

As 2/5 sham-thymectomized mice produced TL antibody when immunized with lymph node cells (Table I), it appears that lymph nodes, as well as spleen and bone marrow, contain some thymocyte precursors; but the point needs more thorough investigation.

**SUMMARY**

TL- mice make TL antibody when immunized with spleen or bone marrow cells from TL+ donors, despite the fact that these cells do not express TL antigen. This has been shown to depend on maturation of TL- precursors, contained in the inoculum, into TL+ cells under influence of the recipient's thymus; the differentiated TL+ cells then evoke production of TL antibody.

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