**Brief Report**

**Graft-Versus-Host Reaction in F1 Hybrid Mice Injected with Pre-Immunized Parental Thymus Cells**

By Luciano Fiore-Donati, Luigi Chieco-Bianchi, Giuseppe De Benedictis and Giuseppe Tridente

Although the thymus has been proved to play an essential role in the development and maintenance of immune reactivity, the mechanisms by which it exerts these functions are still to be elucidated. While significant titers of antibody\(^1,2\) or plasma cell accumulation\(^3,4\) have not been detected in the intact thymus of animals given antigens at distant site, histologic signs indicative of local antibody formation have been observed after direct injection of antigens into the thymus gland.\(^5\) The existence of a functional blood-thymic barrier which excludes thymus cells from exposure to circulating antigens has therefore been postulated.\(^5\) However, whether the immunologic inadequacy of intact thymus depends on its physiologic sequestration or on the relative incapability of thymus cells to take part by themselves in immune reactions remains in considerable doubt. Parental thymus grafted as solid tissue in F1 hybrids does not produce runt disease,\(^6\) but dissociated thymus cells are capable of inducing graft-versus-host reactions, though less effective than other lymphoid cells, when injected into normal or irradiated\(^7\) F1 recipients as well as into newborn mice of unrelated strains.\(^8,9\) However, injection of allogeneic thymus cells in chick embryos\(^10\) or in newborn rats\(^11\) was found to be ineffective or only slightly active in determining graft-versus-host reaction.

The present experiments were designed to study the capacity of thymus cells to initiate a graft-versus-host reaction and particularly to ascertain whether previous sensitization against foreign transplantation antigens would result in a detectable activation of the immunologic potentiality of thymus cells. The Simonsen's method of spleen assay was used as a sensitive test to evaluate the capability of thymus cells derived from normal or pre-immunized parental donors to initiate a graft-versus-host reaction in young F1 hybrid hosts.

**MATERIALS AND METHODS**

*Animals.* Litters of 8 to 10-day-old (C3Hf/Gs x DBA/2)F1 hybrid mice were used as recipients. In all the experiments each litter was divided into 3 groups of at least 2 animals each. One group was kept as uninjected control whereas the others received intraperitoneal injections of either 30 x 10^6 thymus cells or 15 x 10^6 spleen cells derived from 2 to 3-month-old C3Hf/Gs mice. In experiment 1 the test animals were injected with thymus or spleen cells derived from normal C3Hf/Gs donors. In experiment 2 thymus or spleen cells were obtained from C3Hf/Gs donors pre-immunized with DBA/2 spleen cells. To exclude that mediastinal lymph nodes inadvertently removed with thymus could...
**GRAFT-VERSUS-HOST REACTION WITH THYMUS CELLS**

Table 1.—*Graft-Versus-Host Spleen Assay of Thymus and Spleen Cells from Normal or Pre-immunized C3Hf/Gs Donors in Young (C3Hf/Gs x DBA/2)F1 Hybrids*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of Litters Used</th>
<th>Type of Cells Injected</th>
<th>Donor Treatment</th>
<th>No. Cells Injected (x 10⁶)</th>
<th>Mg. Spleen 100 Gm. Mouse</th>
<th>Mean Spleen Index</th>
<th>GVH Reaction*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>None</td>
<td>None</td>
<td>601 ± 105</td>
<td>—</td>
<td>0/6 (0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thymus</td>
<td>None</td>
<td>700 ± 160</td>
<td>1.15</td>
<td>9/10 (100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen</td>
<td>None</td>
<td>1084 ± 188</td>
<td>0/1 (0%)</td>
<td>12/12 (100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>None</td>
<td>None</td>
<td>619 ± 123</td>
<td>—</td>
<td>0/10 (0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thymus</td>
<td>Pre-I</td>
<td>1083 ± 208</td>
<td>1.74</td>
<td>20/22 (91%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen</td>
<td>Pre-I</td>
<td>1612 ± 229</td>
<td>2.62</td>
<td>17/18 (94.5%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>None</td>
<td>None</td>
<td>568 ± 74</td>
<td>—</td>
<td>0/6 (0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thymus§</td>
<td>Pre-I</td>
<td>983 ± 168</td>
<td>1.69</td>
<td>15/17 (88%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen</td>
<td>Pre-I</td>
<td>1760 ± 231</td>
<td>3.08</td>
<td>14/14 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± Standard Deviation.
†GVH = Graft-Versus-Host reaction. Number of mice with spleen index ≥1.30.
§Cell suspension prepared from thymuses removed under dissecting microscope.

have contaminated the thymus cell preparations, the whole experiment with pre-immunized organs was repeated (experiment 3) using thymus glands separated with the aid of a dissecting microscope. Ten days after the inoculation the hybrids were killed, the body and spleen weights were determined and the spleen index calculated. Pre-immunization of C3Hf/Gs donors was obtained by intraperitoneal inoculation of 15 x 10⁶ spleen cells derived from adult DBA/2 mice. Ten days after immunization the animals were killed and spleen and thymus suspensions prepared.

Cell suspensions. Spleen or thymus of donor mice were cut into small fragments with scissors and passed through a fine-meshed stainless steel sieve. Cell suspensions were made in cold physiologic saline, counted in a hemocytometer after staining with Trypan blue and injected intraperitoneally as 0.2 ml. containing the desired amount of unstained nucleated cells.

*Spleen index calculation.* The spleen index was calculated by dividing the mean relative spleen weight of F₁ hybrids receiving spleen or thymus cells by the mean relative spleen weight of uninjected litter-mate controls.

**RESULTS**

The results of the experiments, summarized in table 1, show that a remarkable spleen enlargement was observed in (C3Hf/Gs x DBA/2)F₁ hybrids injected with parental thymus cells derived from C3Hf/Gs donors that had been pretreated with allogeneic DBA/2 cell suspensions (index: 1.74), whereas the spleen weight of hybrids receiving thymus cells from non-sensitized donors showed no significant increase (index: 1.15). A quantitative difference was also found between normal and sensitized spleen cells, the latter being more effective in inducing spleen enlargement in the hybrid recipients (index: 1.74 and 2.62, respectively). It must be noted, however, that thymocytes from pre-immunized donors exhibited lower immunologic capacity than spleen cells from the same donors, in spite of the double cell dosage employed. Entirely comparable results were obtained when thymus cell suspensions were prepared from thymuses carefully removed under the dissecting microscope to avoid possible contamination with cells of mediastinal lymph nodes (index: 1.69).
DISCUSSION

Cohen et al.9 recently reported that allogeneic thymus cell suspensions from adult or newborn C57BL normal donors induced splenomegaly when injected into newborn mice of the A strain. The results of the experiments presented here demonstrate that thymus cells exhibited immunologic activity, as revealed by the capacity of inducing spleen enlargement in F1 hybrids, only when derived from parental donors that had been sensitized previously against the antigens of the other parental strains. Differences in the host/donor strain combinations and/or in cell dosage in relation to the body weight of recipients may account for the discrepancy between these results on the immunologic effectiveness of normal thymus cells. The immunologic activity of thymus cell suspensions from pre-immunized donors observed in our experiments could be due to the presence of lymphoid cells sensitized in peripheral organs and then migrated to the thymus. Alternatively, the present findings may be interpreted by assuming that the injected sensitizing cellular antigens had gained entrance into the thymus in spite of the supposed shielding of the blood-thymic barrier. The latter hypothesis is consistent with recent observations showing that particulate materials administered systemically to adult or newborn mice are found largely distributed within the thymus.12,13 Furthermore, specific recall antibody responses were obtained by transfer of mouse thymus cells from pre-immunized donors to isogenic or allogeneic irradiated recipients.14

However, the experimental data so far available clearly indicate that cells possessing immunologic properties are present in the thymus. The lower activity of thymus cells as compared with cells from other lymphoid organs is presumably due to quantitative rather than qualitative differences in cell population of immunologically competent cells. This is also suggested by recent reports showing that 5–10 x 10^6 thymus cells are relatively ineffective in restoring the immune reactivity of neonatally thymectomized mice,15 whereas a definite immunologic reconstitution can be achieved by injecting as many as 100–200 x 10^6 thymocytes.16

SUMMARY

Dissociated thymus cells are capable of initiating graft-versus-host reaction in (C3Hf/Gs x DBA/2)F1 hybrids only when derived from parental donors previously sensitized against the antigens of the other parental strain. The lower immunologic activity of thymus cells as compared with other lymphoid cells is presumably due to quantitative rather than qualitative differences in immunologically competent cells.

SUMMARIO IN INTERLINGUA

Dissociate cellulas de thymo es capace a initiari un reaction graffo-hospite in muses hybrida (C3Hf/Gs × DBA/2)F1 solmente quando illos es derivate ab parentes-donator previemente sensibilisate contra le antigenos del altere linea parental. Le reducite activitate immunologic de cellulas de thymo in compara-
tion con altere cellulas lymphoide es presumitemente debite a quantitative plus tosto que qualitativa differentias in immunologicamente competente cellulas.

REFERENCES


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