Cyclophosphamide-induced Chimerism

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SUMMARY

BALB/c mice were injected with cyclophosphamide (Cytoxan) (180–280 mg/kg) i.p., and with 3 × 10⁷ (BALB/c ñ × DBA ñ ) F₁ (CDF) spleen cells i.v. The recipients were bled periodically and their sera tested for DBA γ-globulin. All mice had levels of donor γ-globulin at 2 weeks; 17/24 still had detectable levels at 6 months. A greater proportion of mice had detectable donor γ-globulin for a longer time and in higher titer when treated with the highest dose of Cytoxan. All mice received CDF skin grafts 2 weeks after Cytoxan and CDF spleen infusion. Marked prolongation of skin graft survival was observed. In general, skin graft rejection correlated well with loss of donor γ-globulin. However, 4 mice which rejected skin grafts within one month continued to have detectable donor γ-globulin for 6 months. The results are similar to those reported for X-irradiation-induced chimeras.

INTRODUCTION

Lethally X-irradiated mice injected with allogeneic bone marrow or spleen cells become hematopoietic chimeras and readily accept donor type skin grafts (1, 5, 12). The recent development of methods for the identification of mouse γ-globulin allotypes (9) provides an excellent marker for donor lymphoid cells in an immunologically suppressed host. Herzenberg and Cole (8) were the first to demonstrate the persistence of donor γ-globulin in chimeras as late as 9 months after X-irradiation and marrow transplantation. Warner et al. (14), in a methodical study of chimerism induced by lethal or sublethal X-irradiation, found that donor γ-globulin persisted even when donor skin has been rejected. This dissociation has been confirmed by Halpern and Glynn (7).

Recently, an experimental immunotherapeutic model for the treatment of virus-induced murine lymphomas has been developed (J. P. Glynn, B. L. Halpern, and A. Fefer, manuscript in preparation). This entails the use of allogeneic lymphoid cells sensitized against tumor-associated antigens. It necessitates an abrogation of the host's immunologic response, the establishment of chimerism and the serial determination of the persistence of the chimeric state. Cyclophosphamide (Cytoxan), an antineoplastic agent effective in animals and man, has been shown to depress the humoral antibody response to bacterial antigens (2) and the cellular response to skin (3, 6, 13) or tumor homografts (10). As a prelude to the development of the immunotherapy model, the efficacy of Cytoxan in the induction of chimerism with allogeneic lymphoid cells was investigated. The serial detection of allotypic spleen cells was used as the prime measure of persistence of donor lymphoid cells, and was substantiated by skin grafting experiments.

MATERIALS AND METHODS

Mice. Male BALB/c and (BALB/c ñ × DBA/2 ñ ) F₁ (CDF) mice, 10–12 weeks old and 20–25 gm in weight, were obtained from the production colonies of Microbiological Associates, Inc.

Preparation of Cell Suspensions. Spleens were cut into small fragments and pressed through a stainless steel mesh. The cells were then washed X 3 in Hanks' balanced salt solution, centrifuged once at 400 rpm (30 × g) for 5 minutes and twice at 2000 rpm (700 × g) for 5 minutes. The concentration of trypan blue-unstained nucleated cells was determined and adjusted with Hanks' balanced salt solution. The viability of the cell preparations was usually >90%.

Preparation of Antibody against Donor γ-Globulin. Herzenberg et al. (9) demonstrated that the H chain of 7S,γ-globulin of BALB/c mice is antigenically distinct from that of DBA mice, and is determined by alleles for the Ig-1 locus, designated as Ig-l* and Ig-l*, respectively. BALB/c antibody against DBA γ-globulin was obtained by the method of Lieberman and Dray (11). This involved hyperimmunizing BALB/c mice with a washed agglutinate consisting of Proteus mirabilis and serum from DBA/1 mice hyperimmunized against Proteus mirabilis. Sera from BALB/c mice thus immunized were then tested against serially diluted serum from normal CDF mice, by end-point precipitation (11) in a double diffusion system, using an agarose gel. The center well contained BALB/c anti-DBA serum; peripheral wells contained serially diluted CDF serum. Serum from normal CDF mice could always be diluted 1:32 or 1:64 and still yield precipitin lines with the undiluted BALB/c anti-DBA serum. Therefore, the presence of DBA gamma-globulin in experimental BALB/c mice containing CDF lymphoid cells was similarly tested. The result was expressed as the reciprocal of the highest dilution of the test serum which produced a precipitin line with the undiluted BALB/c anti-DBA serum.

Skin Grafting. Skin grafting was performed according to the technic of Billingham and Medawar (4). Bandages were removed on the ninth day. Scab formation and disappearance of the graft was the end-point for graft rejection.
RESULTS

Cytoxan-induced Chimerism as Measured by Persistence of Donor Gamma-Globulin. BALB/c mice were injected intraperitoneally with one of three doses of Cytoxan. Four hours later, each mouse was injected intravenously with 3 × 10^7 trypan blue-unstained nucleated spleen cells from normal adult CDF mice. All recipients were bled periodically and their sera tested for DBA gamma-globulin. Table 1 presents the results in terms of the number of mice with detectable donor gamma-globulin and the mean reciprocal of the gamma-globulin titer in positive mice, as a function of time after treatment with Cytoxan. Two weeks after treatment all 31 recipients exhibited detectable donor gamma-globulin. Cumulatively, 17/24 recipients still had detectable titers of donor gamma-globulin as late as 6 months after Cytoxan and spleen infusion. However, whereas 4/4 mice injected with the highest dose of Cytoxan were positive at 6 months, only 7/12 mice injected with the lowest dose of Cytoxan were positive at that time. The dose of Cytoxan appeared to affect not only the number of recipients exhibiting donor gamma-globulin, but also the titer of the gamma-globulin as a function of time. Mice treated with the highest dose of Cytoxan generally exhibited somewhat higher titers of donor gamma-globulin than did those treated with the lowest dose. It should be emphasized that donor gamma-globulin was never detected in control BALB/c mice inoculated only with CDF spleen cells without Cytoxan pretreatment and tested at the same times as the experimental mice.

Donor Skin Graft Survival in Cytoxan-induced Chimeras. The BALB/c mice whose allotypic data were presented above, received CDF skin grafts 2 weeks after the injection of Cytoxan and CDF spleen cells. Table 2 presents the survival time of these grafts, as compared to that of skin grafted onto untreated controls or onto control mice treated with only spleen cells or only Cytoxan. Pretreatment with Cytoxan and donor spleen cells resulted in markedly prolonged survival of donor skin grafts. 8/30 grafts were still intact at 6 months.

Relationship between Skin Graft Survival and Persistence of Donor Gamma-Globulin. The 8 mice which still retained skin grafts at 6 months, had donor gamma-globulin at that time. Indeed, at any point in time, whenever a donor skin graft was intact, donor gamma-globulin was always detectable in the host serum. However, skin rejection was often, but not always, followed closely by a loss of donor gamma-globulin.

### Table 1

<table>
<thead>
<tr>
<th>Weeks after treatment</th>
<th>Cytoxan dose (mg/kg)</th>
<th>Number of sera tested</th>
<th>Number of sera positive for DBA gamma-globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>13/13 (64)</td>
<td>11/11 (64)</td>
<td>7/7 (64)</td>
</tr>
<tr>
<td>4</td>
<td>12/13 (52)</td>
<td>11/11 (64)</td>
<td>7/7 (64)</td>
</tr>
<tr>
<td>6</td>
<td>10/13 (39)</td>
<td>10/11 (57)</td>
<td>7/7 (64)</td>
</tr>
<tr>
<td>9</td>
<td>8/12 (26)</td>
<td>7/9 (46)</td>
<td>7/7 (51)</td>
</tr>
<tr>
<td>13</td>
<td>7/12 (21)</td>
<td>7/9 (28)</td>
<td>5/5 (53)</td>
</tr>
<tr>
<td>26</td>
<td>7/12 (3)</td>
<td>6/8 (5)</td>
<td>4/4 (14)</td>
</tr>
</tbody>
</table>

Persistence of DBA gamma-globulin in sera of BALB/c mice inoculated with Cytoxan and CDF spleen cells.

* Mean reciprocal titer of positive sera in parentheses.

### Table 2

<table>
<thead>
<tr>
<th>No. of mice</th>
<th>Pretreatment</th>
<th>Median survival time of skin grafts, in days (range)</th>
<th>No. of mice with intact grafts on Day 155</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spleen cells</td>
<td>Cytoxan dose (mg/kg)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>NT</td>
<td>NT</td>
<td>0/6</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>NT</td>
<td>0/11</td>
</tr>
<tr>
<td>6</td>
<td>NT</td>
<td>180</td>
<td>0/6</td>
</tr>
<tr>
<td>2</td>
<td>NT</td>
<td>224</td>
<td>20 (18-21)</td>
</tr>
<tr>
<td>3</td>
<td>NT</td>
<td>280</td>
<td>21 (18-29)</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>180</td>
<td>53 (16 -&gt;180)</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>224</td>
<td>151 (21 -&gt;180)</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>280</td>
<td>&gt;155 (14 -&gt;180)</td>
</tr>
</tbody>
</table>

Prolonged survival of CDF skin grafts on BALB/c mice pretreated with Cytoxan and CDF spleen cells. NT, not treated; +, treated.

1 mouse died with intact grafts on Day 48.
3 mice died with intact grafts on Days 34, 44, and 155.
2 mice died with intact grafts on Day 57 and one on day 155.

Four mice which received spleen cells and Cytoxan rejected donor skin in less than a month, but still had detectable donor gamma-globulin at 6 months.

DISCUSSION

The immunodepressive effect of Cytoxan in murine homograft systems is variable and appears to be strongly dose and time dependent (3). The rejection of F1 cells by parental hosts constitutes a relatively simple model for studying the depression of immunologic reactivity, without the complicating factor of Graft-versus-Host reactions. The data presented clearly show that a single Cytoxan inoculation is capable of preventing or delaying the rejection of allogeneic lymphoid cells. The survival of F1 donor skin grafts was markedly prolonged by pretreating parental hosts with Cytoxan and F1 spleen cells.

Recent progress in the typing of gamma-globulins of different mouse strains (9) has provided a convenient test for the persistence of donor lymphoid cells in the host. The use of this serum protein marker in studies of X-irradiation-induced chimerism by Herzenberg and Cole (8) stimulated the application of this test to Cytoxan-induced chimerism. Serial determinations of donor gamma-globulin revealed that the 3 doses of Cytoxan were quite capable of inducing and maintaining the chimeric state for as long as 6 months. Furthermore, the data suggest a direct relationship between the dose of Cytoxan employed, the frequency of mice with detectable donor gamma-globulin at 6 months, and the mean titer of the donor gamma-globulin when present.

In general, skin graft rejection correlated well with loss of donor gamma-globulin. However, 4 mice which rejected donor skin within one month continued to have detectable levels of donor gamma-globulin for 6 months. This is consistent with the dissociation of the 2 parameters reported by Warner et al. (14) and Halpern and Glynn (7) in X-irradiation-induced chimerism. The possible explanations for this dissociation have been discussed in the 2 reports. The data presented cannot distinguish among the possibilities, but simply confirm the existence of the phenomenon in drug-induced chimerism.
ACKNOWLEDGMENTS

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REFERENCES

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