T Cell Regulation of Pokeweed Mitogen-induced Polyclonal Immunoglobulin Production in Mice: Role of Virus-replicating T Cells in Suppression

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INTRODUCTION

It is well-known that a subpopulation of T cells which can replicate vesicular stomatitis virus (VSV) are generated after the stimulation of lymphocytes by antigens or mitogens. Although the detection and the characterization of these T cells have been studied in various experimental systems, the detailed analysis of functional natures of these T cells is still insufficient. In another series of work, we showed that the antigen-reactive T cells capable of replicating VSV were generated in the antigen-stimulated cultures of spleen cells from mice immunized with sheep erythrocytes, or of lymph node cells from mice sensitized with an epicutaneous application of dinitrofluorobenzene. Attempts to clarify the functional natures of these antigen-activated, VSV-replicating T cells indicated that they represented a T-cell subpopulation(s) involved in the suppression of antibody response, or delayed-type hypersensitivity (DTH). In DTH, it was further shown that VSV-replicating T cells were not the precursors nor the effectors of suppressor T cells, but a subset of T cells indispensable for the generation of suppressor T cells.

The present work was undertaken to investigate the function of VSV-replicating T cells in the regulation of antibody response. For this, the system of pokeweed mitogen (PWM)-induced polyclonal immunoglobulin production was used as an in vitro model of antibody response, since this mitogen effectively stimulates both T and B cells and the T-cell regulation of the B-cell response has several features in common with the antigen-specific response in experimental animals. The results of this study indicate that PWM-induced polyclonal immunoglobulin production of mouse spleen cells is regulated either positively or negatively by T cells. Helper activity was attributed to both Lyt-1+2- and Lyt-1+2+ cells, whereas suppressor T cells were Lyt-1-2+. Virus-replicating T cells in this system were shown to be Thy-1+, Lyt-1+2-. These cells, however, were unlikely to be helper T cells, but may be the inducer of suppressor T cells.

Abbreviations: Ig, immunoglobulin; PFC, plaque forming cells; PFU, plaque forming units; PWM, pokeweed mitogen; Ttv, T cells which can replicate vesicular stomatitis virus; V-PFC, virus plaque forming cells; VSV, vesicular stomatitis virus; DTH, delayed-type hypersensitivity.

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MATERIALS AND METHODS

Methods for lymphocyte preparation, the enrichment of T cells using nylon wool column and
the detection of Ig producing cells as plaque forming cells (PFC) using protein A-coated sheep
erythrocytes as target cells are all as previously described\(^3,11\).

**Animals**

BALB/c mice were obtained from the breeding colony in our Institute. All mice were used
at 10 to 12 weeks of age.

**Reagent**

PWM was purchased from Grand Island Biological Co., Grand Island, N. Y. Protein A from
Staphylococcus aureus was purchased from Pharmacia Fine Chemicals Uppsala, Sweden. N-N’-
diacetyl chitobiose was purchased from Sigma Chemical Co., St. Louis, Mo.

**Anti-Thy-1 and anti-Lyt antibodies**

Monoclonal anti-Thy-1.2 antibody from clone F7D5 was purchased from Olac Ltd. (Oxon,
England). To obtain the B cell enriched fraction, a suspension of spleen cells (10^7 cells/ml) in
MEM containing 2% fetal calf serum (FCS, Grand Island Biological Co.) was incubated for
45 min at 37°C with a 1 : 5,000 dilution of anti-Thy-1.2 antibody plus guinea pig complement
(C, final dilution, 1 : 10). The cells were washed once with MEM, and then the same treatment
was repeated again to ensure that T cells were completely removed.

Arsanilate-conjugated monoclonal anti-Lyt-1 and anti-Lyt-2 antibodies against the frame­
work portion of mouse Lyt antigens were kindly donated by Dr. K. Okumura (University of
Tokyo). Rabbit anti-arsanilate antibody was purchased from Becton Dickinson and Co.
(Mountain View, Ca.). Nylon wool nonadherent spleen cells (10^7) were incubated with 2.5 μg
of anti-Lyt antibodies at 4°C for 30 min in a 1 ml volume of TC medium 199 (Difco Laboratories,
Detroit, Miss.) containing 2% FCS. Cells were washed once with medium 199, and incubated
in the presence of 2 μg/ml of anti-arsanilate antibody for 30 min at 4°C. After centrifugation,
selected rabbit C was then added at a dilution of 1 : 40 and incubated for 30 min at 37°C, and
the remaining cells were washed 3 times with MEM before use.

**Cell culture**

Click’s medium\(^13\) supplemented with 0.25% normal mouse serum and 0.25% horse serum
(Res. Found. Microbial Dis., Osaka University, Osaka, Japan) was used for all cell cultures. For
PWM-induced Ig production, 3 to 5 × 10^6 cells were cultured in a round bottom microtest plate
(A/S Nunc, Roskilde, Denmark) in a 0.2 ml of culture medium containing 10 μg/ml of PWM at
37°C in a humidified atmosphere of 8% CO\(_2\) in air.

For the induction of suppressor T cells, 5 × 10^6 nylon wool nonadherent splenic T cells were
cultured with PWM (3.3–33 μg/ml) in a multiwell culture plate (Linbro, 76–033–05) for 3–4 days
under the same conditions as above. Cells were harvested and washed 3 times with MEM, and
then incubated for 45 min at 4°C in MEM containing 0.1 M N-N’-diacetyl chitobiose to remove
cell-bound PWM\(^13\). Cells were washed 3 times with MEM before use.

**Virus**

Indiana strain of VSV was obtained from the National Institute of Animal Health (Tokyo,
Japan). The virus was grown in L-cells and stocked at −70°C. Rabbit anti-VSV antiserum
was a gift from Dr. G. Tokuda (National Institute of Animal Health, Tokyo, Japan).

**Virus plaque assay**

Virus plaque assay was performed as previously described. Briefly, cells cultured in the presence or absence of PWM were harvested, washed by centrifugation, and portions of $2 \times 10^6$ cells were resuspended in 0.2 ml of culture medium containing $10^8$ plaque forming units (PFU) of VSV. The cell suspension was incubated at 37°C for 2 hr in a humidified air-7% CO$_2$ incubator for virus adsorption, and then washed 4 times. Afterwards they were incubated with anti-VSV serum at 4°C for 1 hr to inactivate free viruses. After four washings $10^3$ or $10^4$ of infected cells were seeded on a monolayer of L-cells to enumerate the virus-replicating cells. Each sample group was tested in triplicate. The dishes were incubated at 37°C in a humidified air-7% CO$_2$ incubator for 1–2 days, and stained with neutral red.

**RESULTS**

**Generation of V-PFC in PWM-stimulated culture of spleen cells**

Spleen cells ($5 \times 10^6$) were cultured in the absence or presence of various doses of PWM. The cells were harvested 1, 2, 3, 4 or 5 days later, and virus replicating cells (V-PFC) were enumerated on L-cell monolayers. The results are shown in Fig. 1. In all doses, the peak response was attained 2 to 3 days after culture. The dose of PWM that induced maximum V-PFC was within the range 25–50 μg/ml.

**Augmentation of polyclonal Ig production by VSV**

Effect of VSV on the polyclonal Ig production was examined by inoculating VSV into PWM-stimulated culture of spleen cells. Spleen cells ($3 \times 10^5$) were cultured in the presence of 10 μg/ml of PWM. In the experiment shown in Fig. 2a, varying doses ($1.5-240 \times 10^5$ PFU) of VSV were added 2 days after the initiation of culture, and in the experiment shown in Fig. 2b, $6 \times 10^5$ PFU of VSV were added 1, 2, 3, 4 and 5 (3 hr before the cell harvest for the protein A plaque assay) days after the initiation of culture. Ig producing cells were assayed as protein A PFC on the 5th day of culture, and the results were expressed as percent of PFC response of control group receiving no VSV. As seen in Fig. 2a, the response was conspicuously augmented.

![Figure 1](image-url)  
*Figure 1*. Generation of VSV-replicating cells in PWM-stimulated culture of spleen cells. Spleen cells ($5 \times 10^6$/ml) were cultured in the absence (○) or presence of 1 (●), 10 (□), 25 (■), 50 (△) or 100 (▲) μg/ml of PWM. Cells were harvested on day 1, 2, 3, 4 and 5, and V-PFC were enumerated on L-cell monolayers as described in Materials and Methods. Values represent the arithmetic mean of triplicate cultures.
Figure 2. Augmentation of Ig production by VSV. (a) Spleen cells (3 × 10⁶/0.2 ml) were cultured in the presence of PWM (10 μg/ml), and varying numbers of VSV (1.5–240 × 10⁵ PFU in 20 μl of culture medium) were added 2 days after the initiation of culture. The number of Ig producing cells was determined on day 5. Control culture received medium only. Results are expressed as a percentage of PFC response of control group. (b) Spleen cells (3 × 10⁶) were cultured in the presence of PWM (10 μg/ml) and 6 × 10⁵ PFU of VSV were added on day 1, 2, 3, 4 or 5 (3 hr before the cell harvest for PFC assay). Cells were harvested on the fifth day of culture and Ig producing cells were enumerated. Control culture received medium only. Results are expressed as a percentage of PFC response of control group.

by the addition of relatively low doses of VSV on the 2nd day of culture, though the inoculation of the highest dose of VSV resulted in the reduction of response. Fig. 2b shows that the addition of VSV 1–3 days after the initiation of culture augmented the response, whereas the response was unaltered when VSV was given 4th or 5th day of culture. These results suggest that there exists in the PWM-induced polyclonal Ig production a dynamic balance between helper and suppressor cells, and that VSV infection abolished the suppressor cell activity. To clarify these points, characterization of the Lyt phenotype of helper and suppressor cells as well as virus replicating T cells was performed in the following sections.

Lyt phenotype of helper T cells

Nylon wool nonadherent spleen cells were treated with anti-Thy-1.2, anti-Lyt-1 or anti-Lyt-2 antibody plus C and then 2 × 10⁵ viable cells were cultured together with 3 × 10⁵ B cells in the presence of PWM. The number of Ig producing cells was determined on the 5th day of culture and the results are shown in Fig. 3. Anti-Lyt-1 plus C completely removed the helper activity of T cells as effectively as anti-Thy-1 plus C treatment. It was also shown that anti-Lyt-2 plus C
Figure 3. Lyt phenotype of helper T cells. Nylon wool nonadherent spleen cells were treated with C alone, or with anti-Lyt-1, anti-Lyt-2, or anti-Thy-1.2 antibody plus C. After treatment, $2 \times 10^5$ viable cells were mixed with $3 \times 10^5$ splenic B cells, and cultured in the presence of PWM (10 $\mu$g/ml). Cells were harvested on day 5, and assayed for the Ig producing cells. The arithmetic mean of triplicate cultures $\pm$ SE is shown.

reduced helper activity by about 50%. These results suggest that the helper function in PWM-induced polyclonal Ig production is mediated not only by Lyt-1$^{+}$2$^{-}$ T cells but also by Lyt-1$^{+}$2$^{+}$ T cells.

**Induction and characterization of suppressor T cells induced by high doses of PWM**

Although the involvement of suppressor cells in the PWM-induced B cell response was shown in human PWM system, it is still uncertain in mice whether the response is actually regulated by the suppressor T cells as in the antibody response to conventional antigens, since the suppressor T cells were not directly demonstrated. Therefore, we attempted to generate suppressor T cells by culturing purified T cells with PWM. For this experiment, $5 \times 10^6$ nylon wool nonadherent splenic T cells were cultured in the absence or presence of various doses of PWM for 3 days then harvested and treated with 0.1 M N-N'-diacetyl chitobiose to remove cell-bound PWM.

<table>
<thead>
<tr>
<th>Group</th>
<th>Assay culture</th>
<th>Precultured T cells*</th>
<th>Addition (1x10^6)</th>
<th>Dose of PWM in preculture (ug/ml)</th>
<th>PFC/well (day 5)</th>
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<tbody>
<tr>
<td>1</td>
<td>B $^\dagger$</td>
<td>--</td>
<td>--</td>
<td></td>
<td>70 ± 20 $^\ddagger$</td>
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<tr>
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<td>B</td>
<td>+</td>
<td>0</td>
<td></td>
<td>2,651 ± 77</td>
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<tr>
<td>3</td>
<td>B</td>
<td>+</td>
<td>3.3</td>
<td></td>
<td>1,393 ± 94</td>
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<tr>
<td>4</td>
<td>B</td>
<td>+</td>
<td>10.0</td>
<td></td>
<td>1,456 ± 95</td>
</tr>
<tr>
<td>5</td>
<td>B</td>
<td>+</td>
<td>33.0</td>
<td></td>
<td>788 ± 53</td>
</tr>
<tr>
<td>6</td>
<td>B $^\ddagger$ T</td>
<td>--</td>
<td>--</td>
<td></td>
<td>1,615 ± 74</td>
</tr>
<tr>
<td>7</td>
<td>B $^\ddagger$ T</td>
<td>+</td>
<td>0</td>
<td></td>
<td>2,746 ± 177</td>
</tr>
<tr>
<td>8</td>
<td>B $^\ddagger$ T</td>
<td>+</td>
<td>3.3</td>
<td></td>
<td>2,573 ± 132</td>
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<tr>
<td>9</td>
<td>B $^\ddagger$ T</td>
<td>+</td>
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<td>831 ± 62</td>
</tr>
<tr>
<td>10</td>
<td>B $^\ddagger$ T</td>
<td>+</td>
<td>33.0</td>
<td></td>
<td>611 ± 96</td>
</tr>
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</table>

* Splenic T cells were cultured in the absence or presence of various doses of PWM for 3 days, and treated with N-N'-diacetyl chitobiose.

$^\dagger$ B, $3 \times 10^6$ B cells, T, $1 \times 10^5$ non-irradiated T cells.

$^\ddagger$ Arithmetic mean of triplicate culture ± SE.
These T cells ($1 \times 10^5$) were mixed with $3 \times 10^5$ B cells or with $3 \times 10^5$ B cells plus $1 \times 10^6$ normal T cells, and cultured for 5 days in the presence of the optimal concentration of PWM (10 μg/ml) before Ig producing cells were enumerated. Table 1 shows that preculturing of T cells in the absence of PWM increased their helper activity (compare group 2 with group 6) while helper activity was decreased by culturing with PWM (compare groups 3, 4 and 5 with group 2). The lowest helper activity was seen in the group cultured with the highest concentration of PWM (group 5). Furthermore, it was shown that T cells precultured with higher concentrations (10 μg/ml or 33 μg/ml) of PWM suppressed the generation of helper activity from fresh T cells (compare groups 9 and 10 with group 5), while T cells precultured with a low concentration (3.3 μg/ml) of PWM did not suppress but rather increased the response (group 8) to the level seen with T cells cultured without PWM (group 7). These results indicate that the optimal doses for the induction of suppressor cells well coincide with that for the generation of virus replicating T cells.

The next experiment explored the Lyt phenotype of suppressor T cells induced by a high dose of PWM. Fresh nylon wool nonadherent splenic T cells or the T cells precultured for 4 days in the presence of PWM (33 μg/ml) were used as a source of suppressor cells. These cells were treated with C alone, anti-Lyt-1 plus C, or anti-Lyt-2 plus C, and added to the assay culture which contained $3 \times 10^5$ B cells and $1 \times 10^5$ T cells irradiated 2,000 R (300 rad/min, 5 mA, 50 kV; Softex Co. Ltd., Tokyo, Japan). The cultures were stimulated with PWM (10 μg/ml) and Ig producing cells were enumerated on day 5. Results are shown in Table 2. The addition of fresh T cells resulted in a reduction of the response which could be circumvented by treating with anti-Lyt-2 plus C but not with anti-Lyt-1 plus C. These results strongly suggested that the suppressor cells were generated from Lyt-1'^-2'^+ cells but not from Lyt-1'^+2'^- cells. Similar results were obtained with T cells precultured with PWM indicating that the suppressor effectors carry the Lyt-1'^-2'^ phenotype.

**Lyt phenotype of VSV-replicating cells**

Spleen cells ($5 \times 10^6$) were cultured for 2 days in the presence of 25 μg/ml of PWM, then harvested and treated with 0.1 M N-N'-diacetyl chitobiose to remove cell bound PWM. These cells were untreated or treated with C alone, anti-Lyt-1 plus C, anti-Lyt-2 plus C or anti-Thy-1.2 plus C, and then the virus plaque assay was performed. Results are shown in Fig. 4.

### Table 2. Lyt phenotype of suppressor T cells

<table>
<thead>
<tr>
<th>Assay culture</th>
<th>T cells added ($1 \times 10^6$)</th>
<th>PWM-stimulated T cells*</th>
<th>Treatment</th>
<th>PFC/well (day 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B+helper T</td>
<td>Fresh T cells</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B+helper T</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>C</td>
</tr>
<tr>
<td>B+helper T</td>
<td>+</td>
<td>-</td>
<td>Anti-Lyt 1+C</td>
<td>316±50</td>
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<tr>
<td>B+helper T</td>
<td>+</td>
<td>-</td>
<td>Anti-Lyt 2+C</td>
<td>2,520±132</td>
</tr>
<tr>
<td>B+helper T</td>
<td>-</td>
<td>+</td>
<td>C</td>
<td>106±50</td>
</tr>
<tr>
<td>B+helper T</td>
<td>-</td>
<td>+</td>
<td>Anti-Lyt 1+C</td>
<td>152±56</td>
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<tr>
<td>B+helper T</td>
<td>-</td>
<td>+</td>
<td>Anti-Lyt 2+C</td>
<td>1,980±60</td>
</tr>
</tbody>
</table>

* Splenic T cells were cultured in the presence of PWM (33 μg/ml) for 4 days, and treated with N-N'-diacetyl chitobiose.

† B, $3 \times 10^5$ B cells, helper T, $1 \times 10^5$ cells irradiated with 2,000 rad.
Suppressor Inducer Cells in Polyclonal Ig Production

Figure 4. Lyt phenotype of VSV-replicating cells. Spleen cells (5 × 10⁶/ml) were cultured for 2 days in the absence or presence of PWM (25 μg/ml), then harvested and treated with 0.1 M N-N'-diacetyl chitobiose. These cells were untreated or treated with C alone, anti-Lyt-1 plus C, anti-Lyt-2 plus C or anti-Thy-1.2 plus C, and V-PFC were enumerated on L-cell monolayers. The number of background V-PFC (V-PFC in unstimulated culture, 12/10⁴ cells) were subtracted from each value before calculation of the mean. Arithmetic mean of triplicate cultures±SE is shown. Replicating cells were eliminated by anti-Lyt-1 plus C treatment to the same extent by anti-Thy-1 plus C treatment, whereas treatment with anti-Lyt-2 plus C did not alter the number of VSV-replicating cells. These results indicate that VSV-replicating cells are not the precursors nor the effectors of suppressor cells, but are the cells which have the same Lyt phenotype as at least a subpopulation of helper T cells. Although it is not clear from this experiment whether the VSV-sensitive cells are identical to the helper T cells or not, the results in the following section strongly suggest that VSV-sensitive cells are different subpopulation of cells from helper cells.

Abolishment of suppressor but not helper activity by VSV infection

Figure 5. Abolishment of suppressor activity by VSV. Nylon wool nonadherent splenic T cells (5 × 10⁶/ml) were cultured for 2 days in the absence or presence of 25 μg/ml of PWM. A portion of PWM-stimulated T cells was infected with VSV, washed and treated with anti-VSV antiserum. Control cells were treated in the same way except for VSV infection. PWM-stimulated and VSV-infected or uninfected cells, or PWM-unstimulated cells were cultured again for 2 days in the absence or presence of PWM (25 μg/ml), then harvested and treated with 0.1 M N-N'-diacetyl chitobiose. These cells (1 × 10⁵) were added to the assay culture which comprised 3 × 10⁵ B cells and 1 × 10⁵ 2,000 R irradiated T cells, and cultured with PWM (10 μg/ml) for 5 days before the number of Ig producing cells was determined. Arithmetic mean of triplicate cultures±SE is shown.
In this section, the effect of VSV-infection on the suppressive activity of T cells precultured with a high dose of PWM were investigated. Nylon wool nonadherent splenic T cells \(5 \times 10^6\) were cultured in the absence or presence of 25 \(\mu\)g/ml of PWM for 2 days then harvested and treated with 0.1 M N-N'-diacetyl chitobiose. A portion of PWM-stimulated T cells were infected with VSV and then thoroughly washed and treated with anti-VSV antiserum. These VSV-infected or, as controls, uninfected cells or PWM-unstimulated cells were further cultured for 2 days before addition into the assay culture which comprised B cells and X-irradiated T cells. To eliminate the possible effect of free virus, anti-VSV antiserum was added into the culture throughout the culture period. Ig producing cells were enumerated on day 5, and the results are shown Fig. 5. Infection by VSV not only eliminated the suppressor activity of PWM-stimulated T cells but rested the helper activity comparable to that of PWM-unstimulated precultured cells. Since suppressive activity is carried by Lyt-1-2+ cells (Table 2), and since VSV preferentially infect and replicate in Lyt-1+2- cells (Fig. 4), these results strongly suggest that the target of VSV may be a subset of Lyt-1+2- cells which are distinct from helper T cells but are indispensable for the generation of suppressor effector cells.

**Cooperative effect of Lyt-1+2- cells on the generation of Lyt-1-2+ suppressor cells**

Although the results of the experiment in the preceding section indicated that the induction of suppressor cells by PWM was prevented by the VSV infection, it has not been directly demonstrated that the cells carrying the Lyt-1+2- phenotype are required for the generation of suppressor cells by PWM. In this section, therefore, the effect of Lyt-1+2- cells on the development of suppressor cells was investigated. A Lyt-1+2- or Lyt-1-2+ population of T cells was isolated by treating nylon wool nonadherent spleen cells with anti-Lyt-2 or anti-Lyt-1 antibody plus C, then \(5 \times 10^6\) unseparated T cells, Lyt-1+2- cells, Lyt-1-2+ cells, or a 1:1 mixture of Lyt-1+2- and Lyt-1-2+ cells were cultured in the presence of PWM (33 \(\mu\)g/ml). After 4 days in culture, cells were harvested and treated with 0.1 M N-N'-diacetyl chitobiose. Cells from a co-culture of Lyt-1+2- and Lyt-1-2+ cells were further treated with anti-Lyt-1 plus C to remove the Lyt-1+2- cells. After these treatments, \(1 \times 10^5\) of each cultured cells were added to \(3 \times 10^5\) B cells and

<table>
<thead>
<tr>
<th>Group</th>
<th>Preculture of T cells*</th>
<th>PWM (33 (\mu)g/ml)</th>
<th>Treatment after culture</th>
<th>Assay culture†</th>
<th>PFC/well (day 5)</th>
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<tbody>
<tr>
<td>1</td>
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<td>B</td>
<td></td>
<td>680±40</td>
</tr>
<tr>
<td>2</td>
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<tr>
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<td>B+helper T</td>
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<tr>
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<td>Anti-Lyt 1+C</td>
<td>B+helper T</td>
<td>1,620±321</td>
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</table>

* Splenic T cells (unseparated), anti-Lyt 2+C treated T cells (Lyt 1), anti-Lyt 1+C treated T cells (Lyt 2), or a 1:1 mixture of Lyt 1 and Lyt 2 were cultured in the presence of PWM (33 \(\mu\)g/ml) for 4 days, and treated with N-N'-diacetyl chitobiose.

† Precultured cells (\(1 \times 10^5\)) were added to the assay culture of \(3 \times 10^5\) B cells (B) plus \(1 \times 10^5\) T cells irradiated with 2,000 rad (helper T).
$1 \times 10^5$ irradiated T cells then stimulated with PWM (10 μg/ml). The number of Ig producing cells was determined on day 5 and the results are shown in Table 3.

PWM-stimulated unseparated T cells were highly suppressive (group 4), conforming the data in Table 2. Weak suppressive activity was induced by prestimulation of Lyt-1-2+ cells (compare groups 6 and 3) but not by prestimulation of Lyt-1+2- cells (group 5). In contrast, strong suppression comparable to that in unseparated T cells was seen with Lyt-1-2+ cells that were prestimulated together with Lyt-1+2- cells (group 7). These results indicate that in the PWM response, Lyt-1+2- T cells amplify the generation of Lyt-1-2+ suppressor cells.

**DISCUSSION**

In this study it was shown that a large number of cells which can replicate vesicular stomatitis virus were generated in PWM-stimulated cultures of normal mouse spleen cells. The Lyt phenotype of these cells was shown to be Thy-1+, Lyt-1+2-. These cells, however, were unlikely to be helper T cells but may be a subset of T cells involved in suppression, since inoculation of VSV into a PWM-stimulated culture of spleen cells resulted in marked augmentation of Ig production, and since the suppressor activity but not helper activity of T cells which had been precultured with a high dose of PWM was abolished by the infection with the virus (Fig. 2 and Fig. 5). It was further demonstrated that the presence of Lyt-1+2- T cells in the culture markedly enhanced the generation of suppressor cells by PWM (Table 3). Using SRBC as the antigen, we have previously shown that the T cells (Tv) which could replicate VSV on activation by the antigen did not represent helper T cells nor T cells mediating DTH, but was suggested to be the cells involved in the suppression of immune responses. Detailed analysis of the function of these cells in DTH revealed that Tv were probably a subset of T cells indispensable for the generation of suppressor T cells. Present study showed that this was also true for PWM-induced polyclonal Ig production.

The VSV-replicating cells induced by PWM-stimulation were found to be Lyt-1+2- (Fig. 4). This result seems inconsistent with the result obtained by Senic and Bloom who showed in secondary mixed leukocyte culture about 70% of VSV-replicating cells were Lyt-1-2- whereas the rest were Lyt-1-2+. Since I have not investigated the Lyt phenotype of VSV-replicating cells in other experimental systems, it is unclear whether PWM stimulation of normal spleen cells is an exceptional case or whether VSV-replicating cells are also Lyt-1-2+ cells in the primary immune response to the conventional antigens whereas some subpopulation(s) of Lyt-1-2+ cells become sensitive to VSV in the hyperimmunization such as secondary mixed lymphocyte culture.

Recently, it is well known that there exist the sequence of cellular interactions in the suppression of immune responses. At least three distinct subpopulation of T cells are shown to be involved in the suppression of DTH, and a similar or more complex cell interactions are shown in the suppression of antibody response. In both cases, the first set of T cells activated by the antigen are Lyt-1+2- non-helper T cells, although it is not clarified whether or not the suppressor pathways for both DTH and antibody response are initiated by the same Lyt-1+2- cells. Our present and previous papers showed that the Lyt-1+2- cells which function as the initiators of the suppression of DTH and those for polyclonal Ig production (and probably also
for antigen-induced antibody response) have a common nature that VSV are selectively replicated in these cells. Further study, however, is required to clarify why this virus is replicated by a restricted subpopulation(s) of T cells, suppressor inducers, while suppressor effector or helper T cells are resistant to the infection with this virus.

**Abbreviations**

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**SUMMARY**

Role of virus-replicating T cells in the regulation of pokeweed mitogen (PWM)-induced polyclonal immunoglobulin (Ig) production was studied in mice. A large number of cells which can replicate vesicular stomatitis virus (VSV) were generated in PWM-stimulated cultures of spleen cells. The optimal dose of PWM for the generation of VSV-replicating cells was within the range 25 to 50 μg/ml, which were also shown to be optimal for the induction of suppressor T cells. In spleen cell cultures, helper activity was attributed not only to Lyt-1+2− but also to Lyt-1+2+ T cells and both the precursor and effector suppressor T cells were found to be Lyt-1−2+. The VSV-replicating cells in this system were shown to be Thy-1+ and Lyt-1+2−. These cells, however, were unlikely to be helper T cells, but may be a subset of T cells involved in suppression. Thus, inoculation of VSV into the PWM-stimulated cultures of spleen cells resulted in the marked augmentation of Ig production, and the suppressive activity of T cells which had been precultured with a high dose of PWM was abolished by the infection with this virus while the helper activity remained unaffected. Further, it was shown that although the Lyt-1−2+ T cell subset could be directly stimulated by PWM to generate suppressor cells, the presence of Lyt-1+2− cells in the culture markedly enhanced the generation of Lyt-1−2+ suppressor cells.

**REFERENCES**

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