HUMAN T CELL ACTIVATION

II. A New Activation Pathway Used by a Major T Cell Population Via a Disulfide-bonded Dimer of a 44 Kilodalton Polypeptide (9.3 Antigen)

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Considerable progress has been made in the delineation of human functional T cell subsets and in the identification of T cell–specific and –associated antigens (reviewed in 1 and 2). Some of these antigens are closely involved in human T cell activation and proliferation. The importance of the T3/Ti (Ti, putative T cell receptor) complex in antigen-specific T cell activation has recently been reviewed (3, 4). Although this is a major activation pathway, an alternative antigen-independent pathway involving the T11 antigen, the sheep erythrocyte receptor, has been proposed (5, 6). Two unique anti-T11 monoclonal antibodies (mAb) in combination were shown to activate T cells by Ca\(^{2+}\) influx, and to induce T cells to proliferate. This pathway was shown to be related to the T3/Ti complex in that modulation of the T3/Ti complex inhibited the effects by these two mAb.

Recently, we demonstrated that anti-T3 mAb, in collaboration with tumor promoter 12-o-tetradecanoyl phorbol-13-acetate (TPA), induced human T cells to express interleukin-2 (IL-2) receptors, to secrete IL-2, and to proliferate (7). This process was independent of monocytes. Herein, other anti-T cell mAb were screened for their effects on T cell activation in this system. mAb 9.3, reactive with a disulfide-bonded dimer of a 44 kilodalton (kD) polypeptide on a major T cell population, was found to induce T cell activation and proliferation in collaboration with TPA, in a manner similar to that of the anti-T3 mAb. Modulation of the T3/Ti complex did not inhibit the effects induced by mAb 9.3 and TPA. Thus, antigen 9.3 is involved in a novel activation pathway used by a major T cell population.

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1 Abbreviations used in this paper: FCS, fetal calf serum; FITC, fluorescein isothiocyanate; HS, human serum; IL, interleukin; mAb, monoclonal antibody; PBS, phosphate-buffered saline; PMC, peripheral mononuclear cells; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SRBC, sheep red blood cell; Ti, putative T cell receptor; TPA, 12-o-tetradecanoyl phorbol-13-acetate.

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Materials and Methods

**Monoclonal Antibodies.** Purified mAb 9.3, 9.6 (T11), 3.5 (T12), 10.2 (Leu-1), 17.2 (T4), and 10.1 (T8) were obtained from ascitic fluids as described previously (8). The assignment of specificities to these antibodies were done by their cellular reactivities and by the molecular weights of the reactive antigens. mAb TE (T11) and 235 (T3) were generated in our laboratory as described (7). Anti-putative T cell receptor (anti-Ti) mAb S511 and C37 were generous gifts from Dr. R. Bigler (The Rockefeller University, NY) and Drs. Y. Buskkin and C. Y. Wang (Sloan-Kettering Cancer Center, NY), respectively.

**Biotinylation of Purified mAb AT-1.** mAb AT-1, directed against the IL-2 receptor, was produced as described previously (9). Biotinylation of purified AT-1 was carried out according to a standard method. 0.5 ml of AT-1 (1 mg/ml) in 0.2 M Tris HCl, pH 8.1, was mixed quickly with 0.1 ml of 3.4 mg/ml of biotin N-hydroxysuccinimide ester (Calbiochem-Behring Corp., San Diego, CA) solution and stirred for 30 min at room temperature. The biotinylated AT-1 was extensively dialyzed against PBS (phosphate-buffered saline).

**Cell Preparation.** Peripheral mononuclear cells (PMC) were isolated from buffy-coats from normal donors or peripheral blood, using Ficoll-Hypaque density gradient centrifugation. To isolate monocyte-free T cells, PMC were further separated by Percoll continuous gradient centrifugation, followed by carbonyl iron treatment, nylon wool column, and plastic adherence (10-12). Monocyte-free T cells were also obtained by sheep red blood cell (SRBC) rosetting (13), carbonyl iron treatment, nylon wool column, and plastic adherence. These T cell preparations contained <0.1% monocytes, by nonspecific esterase staining. SRBC rosetting separation had no effect on the results, although T11 antigen was found to be an alternative pathway of T cell activation (5).

Cells were suspended in complete medium consisting of RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (FCS) (HyClone Laboratories, Logan, UT), 2 mM l-glutamine, and 50 µg/ml gentamicin (Gibco Laboratories). In some experiments, 10% heat-inactivated human AB serum (HS) was used instead of FCS.

**Immunofluorescence Studies.** Cells were stained with mAb at 4°C for 30 min. After extensive washing, the cells were further incubated with FITC-conjugated goat anti-mouse F(ab')2 at 4°C for 30 min. For the study of IL-2 receptor expression, biotinylated AT-1 was used as the first ligand, and FITC-conjugated avidin (Becton Dickinson and Co., Mountain View, CA) was used as the second ligand antibody. After wash, the cells were analyzed with a Coulter Epics V flow cytometer (Coulter Electronics Inc., Hialeah, FL). Integrated fluorescence of the gated population was measured, and 10,000 cells were analyzed.

**Proliferation Assays.** PMC or monocyte-depleted T cells (2 × 10^5 cells) in 200 µl of complete medium were incubated in triplicate in flat-bottomed, 96-well plates at 37°C for 3 d in a 5% CO2 atmosphere. During the last 8 h, cells were pulsed with 0.4 µCi of [3H]thymidine (sp act 75 Ci/mmol; Amersham Corp., Arlington Heights, IL), and incorporated radioactivity was determined as described (14). For proliferation assays, TPA (Sigma Chemical Co., St. Louis, MO), recombinant IL-2, and purified IL-2 and IL-1 (Genzyme, Boston, MA) were used.

**Modulation Studies.** PMC (10^6 cells/ml) or cells of the HPB-ALL leukemic line (2.5 × 10^6 cells/ml) in complete medium were incubated with a saturating dose of mAb for 18 h at 37°C. Cells were washed three times, then stained as described above.

**IL-2 Activity Assay.** 4 × 10^3 murine IL-2-dependent HT-2 cells (a gift from Dr. K. Himeno of the Oklahoma Medical Research Foundation) in 100 µl complete medium, containing 2.5 × 10^-5 M 2-mercaptoethanol were incubated with 100 µl of culture supernatants of various dilutions, in triplicate, at 37°C for 24 h. Then, 0.4 µCi of [3H]-thymidine was added, and incubation was continued for another 4 h. The incorporated radioactivity was assayed as described above.

**IL-2 Production.** T cells (10^6 cells/ml) in complete medium were cultured in the presence of various activators for 12 h. Supernatants were collected by centrifugation, filtered, and assayed for IL-2 activity as described above.
Iodination, Immunoprecipitation and Autoradiography. T lymphocytes (2 × 10⁷) were labeled with ¹²⁵I (sp act 15.8 mCi/µg of iodine, Amersham Corp.) by the lactoperoxidase technique (7). Immunoprecipitation and autoradiography were performed as described (7). For electrophoresis in nonreduced condition, cells were preincubated in PBS containing 10 mM iodoacetamide before lysis, and then lysed in lysis buffer containing 100 mM iodoacetamide.

Results

9.3 and T3/Ti are Distinct Molecules. mAb 9.3 has been shown (15) to be an IgG2a cytotoxic for 50–80% of the peripheral blood T cells. By immunofluorescence, it stained 60–80% of the isolated T cells. Initially, mAb 9.3 was found to precipitate a single polypeptide of 44 kD from Jurkat, a T leukemia cell line (15). In order to ascertain the molecular weight of the 9.3 antigen, peripheral blood T cells were ¹²⁵I-labeled by lactoperoxidase, and immunoprecipitates of mAb 9.3 were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) under both reduced and nonreduced conditions. As shown in Fig. 1, mAb 9.3 precipitated an 88 kD polypeptide under nonreduced conditions, which was reduced to a 44 kD polypeptide.

The relationships among 9.3, T3/Ti, and T11 antigens were investigated.

![Figure 1](image_url)  
**Figure 1.** Immunoprecipitation of 9.3 antigen from ¹²⁵I-T lymphocytes. The precipitates were analyzed by SDS-PAGE (10% and 8% polyacrylamide) under reducing (R) and nonreducing (NR) conditions, respectively. Control immunoprecipitates without mAb 9.3 showed no bands.
mAb 9.3 induced redistribution of its reactive antigen, but modulation was partial. Modulation of the T3 complex from peripheral blood T cells by anti-T3 mAb 235 did not reduce the staining intensity by mAb 9.3. Similarly, partial modulation of the T11 antigen by anti-T11 mAb TE had no effect on the staining by mAb 9.3.

To define the relationship between 9.3 and T3/Ti further, comodulation experiments were carried out using HPB-ALL, a T leukemia cell line, and mAb C37, which identifies the Ti molecule clonotypically on HPB-ALL. As shown in Fig. 2, modulation of the T3 antigen with anti-T3 mAb (235) reduced the staining by anti-T3 mAb and anti-Ti mAb C37 markedly. Despite this almost complete modulation of the T3/Ti complex, staining for 9.3 was not changed. As a control, anti-Leu-1 mAb was included. Similar results were obtained when the T3/Ti complex was modulated with anti-Ti mAb C37 (Fig. 2 C). In another system involving T3+ human leukemic S cells, mAb S-511 was shown to be clonotypic (16). After modulation of the T3 complex by mAb 235, mAb S-511 was no longer reactive with leukemic S cells. mAb 9.3 staining intensity was not reduced by this anti-T3 mAb treatment. Attempts to modulate the T3/Ti complex by mAb S-511 were not successful.

**mAb 9.3 Induced T Cell Proliferation in the Presence of TPA.** mAb 9.3 was not mitogenic either for PMC or for isolated T cells. In the presence of TPA, mAb 9.3 was strongly mitogenic for T cells that were contaminated with <0.1% monocytes (Table I), as well as for PMC (data not shown). The mitogenicity of mAb 9.3 at 1 μg/ml was dependent on the dose of TPA, and its mitogenic effect was apparent even in the presence of 0.3 ng/ml of TPA. Other mAb, such as anti-T11, -T12, -Leu-1, -T4, and -T8 were not mitogenic in this system. mAb
**Table I**

*T Cell Proliferation in Presence of Anti-T Cell mAb and Various Concentrations of TPA*

<table>
<thead>
<tr>
<th>Antibody (1 µg/ml)</th>
<th>[3H]Thymidine uptake at TPA concentrations (ng/ml) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>9.3</td>
<td>208</td>
</tr>
<tr>
<td>T3 (235)</td>
<td>188</td>
</tr>
<tr>
<td>T11 (TE)</td>
<td>255</td>
</tr>
<tr>
<td>T12 (3.5)</td>
<td>150</td>
</tr>
<tr>
<td>Leu-1 (10.2)</td>
<td>235</td>
</tr>
<tr>
<td>T4 (17.2)</td>
<td>175</td>
</tr>
<tr>
<td>T8 (10.1)</td>
<td>214</td>
</tr>
<tr>
<td>Medium</td>
<td>194</td>
</tr>
</tbody>
</table>

Monocyte-depleted T cells (2 × 10⁵) were incubated with 1 µg/ml of mAb in the presence of various concentrations of TPA. [3H]Thymidine uptake was measured after 3 d. The standard deviations of each mean value were within 15%.

**Table II**

*T Cell Proliferation Induced by Various Concentrations of mAb T4 or 9.3 in Presence of TPA*

<table>
<thead>
<tr>
<th>mAb</th>
<th>[3H]Thymidine uptake at antibody concentrations (ng/ml) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>T4 (17.2)</td>
<td>1,225</td>
</tr>
<tr>
<td>9.3</td>
<td>1,249</td>
</tr>
</tbody>
</table>

Monocyte-depleted T cells (2 × 10⁵) were incubated in the presence of various concentrations of mAb T4 or 9.3, and TPA (0.6 ng/ml). [3H]-Thymidine incorporations were determined after 3 d. The standard deviations of each mean value were within 15%.

235, an anti-T3 mAb of IgM isotype was mitogenic in the presence of TPA, and it was included for comparison. mAb 9.3 induced a proliferative response just as vigorous as anti-T3 mAb. Two other experiments showed similar results.

mAb 9.3 was found to be mitogenic, in a dose-dependent manner, in the presence of TPA (Table II). In the presence of 0.6 ng/ml of TPA, as little as 1 ng/ml of mAb 9.3 induced a threefold increase in [3H]thymidine uptake. In the presence of 1,000 ng/ml of mAb 9.3, 66,009 cpm was detected. An anti-T4 (17.2) mAb, reactive to the helper T cells, was included as a control antibody. This antibody had no T cell mitogenic effect in combination with TPA.

The mitogenic effect of mAb 9.3 in the presence of TPA was studied with T cells isolated without the SRBC rosetting procedure. As shown in Table III, the SRBC rosetting procedure had a marginal effect on the mitogenesis in this system. In two other experiments, not shown here, no difference was detected when the two T cell isolation procedures were compared. The effect of FCS in this system was also studied. With lower concentrations of TPA (<1.0 ng/ml),
ACTIVATION OF HUMAN T CELLS BY MONOCLONAL ANTIBODY 9.3

TABLE III
Effects of SRBC Rosetting and FCS on mAb 9.3-induced Mitogenesis

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Serum</th>
<th>mAb</th>
<th>[(^{3}\text{H})]Thymidine uptake at TPA concentrations (ng/ml) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>FCS</td>
<td>Medium</td>
<td>325</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.3</td>
<td>301</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>Medium</td>
<td>262</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.3</td>
<td>293</td>
</tr>
<tr>
<td>B</td>
<td>FCS</td>
<td>Medium</td>
<td>276</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.3</td>
<td>252</td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>Medium</td>
<td>316</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.3</td>
<td>386</td>
</tr>
</tbody>
</table>

In exp. A, T cells were separated by SRBC rosetting, carbonyl iron treatment, nylon wool column, and plastic adherence. In exp. B, T cells were separated by Percoll continuous gradient centrifugation, carbonyl iron treatment, nylon wool column, and plastic adherence. Monocyte-depleted T cells (2 x 10^6) were incubated with or without 1 \(\mu\)g/ml of mAb 9.3 in the presence of various concentrations of TPA in complete media containing either FCS or HS. [\(^{3}\text{H}\)]Thymidine uptake was measured after 3 d. The standard deviations of each mean value were within 15%.

TABLE IV
Effect of Various Concentrations of IL-2 or IL-1 on T Cell Proliferation With or Without mAb 9.3

<table>
<thead>
<tr>
<th>Lymphokine</th>
<th>([^{3}\text{H}])Thymidine incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medium</td>
</tr>
<tr>
<td>U/ml</td>
<td>cpm</td>
</tr>
<tr>
<td>IL-2</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>118</td>
</tr>
<tr>
<td>125</td>
<td>158</td>
</tr>
<tr>
<td>250</td>
<td>297</td>
</tr>
<tr>
<td>500</td>
<td>511</td>
</tr>
</tbody>
</table>

T cells (2 x 10^6) were cultured for 3 d in the presence of various concentrations of IL-1 or IL-2 with or without 1 \(\mu\)g/ml of mAb 9.3. [\(^{3}\text{H}\)]Thymidine incorporation was determined as described in Materials and Methods. The standard deviations of each values were within 15%. IL-2 and IL-1 activities were confirmed using an IL-2-dependent cell line (HT-2), and mouse thymocytes, respectively.

mAb 9.3 was more mitogenic in FCS than in HS. However, at 1.0 ng/ml TPA, mAb 9.3 was equally mitogenic in both culture media.

**IL-1 and IL-2 Did Not Collaborate with mAb 9.3.** Addition of IL-1 or IL-2 to a monocyte-depleted T cell culture in the presence of 1 \(\mu\)g/ml of mAb 9.3 did not induce T cell proliferation (Table IV). In the case of IL-2, as much as 500
U/ml was added to the culture without effect. No attempts were made to see if these interleukins were effective in collaboration with mAb 9.3 to induce T cell proliferation in the presence of monocytes. Both IL-1 and IL-2 preparations were shown to be active as potent stimulators of DNA synthesis of mouse thymocytes in the presence of phytohemagglutinin, and in IL-2-dependent HT-2 cells, respectively.

**T Cell Proliferation Induced by mAb 9.3 Plus TPA Was Associated with IL-2 Receptor Expression and IL-2 Production.** mAb 9.3-induced T cell proliferation in the presence of TPA was associated with IL-2 receptor expression and IL-2 production. In the case of IL-2 receptor expression, biotinylated anti–IL-2 receptor mAb (AT-1) and avidin-conjugated fluorescein isothiocyanate (FITC) were used. As shown in Fig. 3, mAb 9.3 alone did not induce any increase in IL-2 receptor expression. mAb 9.3 and TPA markedly increased IL-2 receptor-positive cells. By 60 h, ~84% of the cells were positive for IL-2 receptor, detectable by mAb AT-1. TPA alone also induced a significant number of T cells to express IL-2 receptors. However, the staining intensity of IL-2 receptor on TPA-treated T cells was much weaker than that on mAb 9.3 plus TPA-treated T cells (Fig. 4).

IL-2 production by mAb 9.3 and TPA-treated T cells was examined with an IL-2-dependent murine T cell line, HT-2. As shown in Table V, T cells treated with either mAb 9.3 or TPA alone did not secrete IL-2. A considerable amount of IL-2 was secreted by T cells treated with mAb 9.3 and TPA. Significant proliferation was seen even when culture supernatants were diluted 1:60. As an added control, the addition of mAb 9.3 to the supernatant of TPA-treated T cells did not support the proliferation of HT-2 cell line.

**T3 Modulation Did Not Significantly Affect T Cell Activation with mAb 9.3 Plus TPA.** The modulation of the T3 complex in 9.3 mAb–induced T cells proliferating in the presence of TPA was studied. Monocyte-depleted T cells were treated with mAb 235 (an IgM anti-T3) for 15 h to induce T3 complex

![Figure 3](image-url)

**Figure 3.** Time course of expression of IL-2 receptors on T cells. Monocyte-depleted T cells (2 x 10⁶) were incubated in the presence of mAb 9.3 (1 μg/ml) with or without TPA (0.6 ng/ml). The percentages of IL-2 receptor–positive cells were determined by biotinylated AT-1 and FITC-avidin at the indicated times. 9.3 plus TPA (O), TPA alone (Δ), 9.3 alone (☐), medium (□). 10,000 cells were analyzed with a Coulter Epics V Flow cytometer, as described in Materials and Methods. [H]Thymidine incorporation (cpm) was as follows: medium (319), 9.3 antibody (173), TPA (1,306), 9.3 plus TPA (38,081).
modulation. mAb 235 modulates T3 complex on T cells in the absence of monocytes (our unpublished observations).

After this treatment, 2–5% of residual T cells remained weakly positive for T3 antigen, as detected by immunofluorescence. These T cells were cultured in the presence of either anti-T3 or mAb 9.3 with TPA to determine the effect of T3 modulation. Anti-T3 plus TPA–induced proliferation of T3-modulated cells was 9 and 28% of that of nonmodulated cells on days 2 and 3, respectively (Table VI). On the other hand, proliferation induced by mAb 9.3 plus TPA in T3-modulated cells was 92 and 85% of that of nonmodulated cells on days 2 and

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**Figure 4.** Flow-cytometric analysis of the expression of IL-2 receptors on T cells. Monocyte-depleted T cells were incubated with medium, mAb 9.3 (1 μg/ml) alone (2), TPA (0.6 ng/ml) alone (3), and TPA plus mAb 9.3 (4). IL-2 receptor–positive cells were analyzed with a flow cytometer as described in Fig. 3. (1) indicates the staining with nonbiotinylated AT-I and avidin-FITC.

**Table V**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>[3H]Thymidine incorporation at various dilutions of culture supernatant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:2</td>
</tr>
<tr>
<td>Medium</td>
<td>1,170</td>
</tr>
<tr>
<td>mAb 9.3 (1 μg/ml)</td>
<td>1,252</td>
</tr>
<tr>
<td>TPA (0.6 ng/ml)</td>
<td>1,149</td>
</tr>
<tr>
<td>TPA + mAb 9.3</td>
<td>6,026</td>
</tr>
</tbody>
</table>

Monocyte-depleted T cells (10⁶/ml) were incubated in the presence of mAb 9.3 (1 μg/ml) with or without TPA (0.6 ng/ml). After 12 h incubation, supernatants were collected and assayed using an IL-2-dependent murine cell line (HT-2), as described in Materials and Methods. The standard deviations of each value were within 15%.
Table VI
Effect of T3 Modulation on T Cell Proliferation Induced by mAb 9.3 Plus TPA

<table>
<thead>
<tr>
<th>Day</th>
<th>Antibody for modulation</th>
<th>[3H]Thymidine uptake when stimulated with TPA and:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Medium (235) (1 µg/ml)</td>
</tr>
<tr>
<td>2</td>
<td>Medium</td>
<td>1,342</td>
</tr>
<tr>
<td></td>
<td>Anti-T3 (235)</td>
<td>1,324</td>
</tr>
<tr>
<td></td>
<td>Anti-Leu-1 (10.2)</td>
<td>1,450</td>
</tr>
<tr>
<td>3</td>
<td>Medium</td>
<td>1,563</td>
</tr>
<tr>
<td></td>
<td>Anti-T3 (235)</td>
<td>4,273</td>
</tr>
<tr>
<td></td>
<td>Anti-Leu-1 (10.2)</td>
<td>1,473</td>
</tr>
</tbody>
</table>

Monocyte-depleted T cells (10⁶ cells/ml) were modulated with medium, anti-T3 mAb (235), or anti-Leu-1 (10.2) at 37°C for 15 h. Cells were washed three times with media and further incubated with medium, anti-T3 (1 µg/ml), or mAb 9.3 (1 µg/ml) in the presence of 0.6 ng/ml TPA for 2 or 3 d. [3H]Thymidine incorporation was determined after an 8-h pulse. The standard deviations of each mean value were within 15%.

3, respectively. Leu-1 modulation did not influence greatly T cell proliferation, either with anti-T3 mAb plus TPA, or with mAb 9.3 plus TPA.

Discussion

The T3/Ti complex and the T11 molecule have been shown (3, 5) to mediate two T cell activation pathways. The T3/Ti pathway is antigen specific and dependent on the presence of monocytes. The T11 pathway is non-antigen specific and independent of monocytes. Despite these differences, these two pathways are related. The T11 pathway is blocked by the modulation of the T3 complex. In this study, a third T cell activation pathway has been identified. This pathway is mediated by a disulfide-bonded dimer of a 44 kD structure identified by mAb 9.3. This bimolecular structure is similar to that of T cell receptor molecule, Ti. However, comodulation experiments with anti-Ti and anti-T3 antibodies, using HPB-ALL cells, showed that the Ti and 9.3 molecules are not the same. In addition, V8 protease digestion of Ti and 9.3 antigens precipitated from surface-iodinated HPB-ALL cells revealed no similar peptides. Furthermore, T cell activation by mAb 9.3, in the presence of TPA, was not affected by T3 modulation. These results suggest that 9.3 activation pathway is different from those via T3 and T11.

This study and our investigation involving anti-T3 antibodies (7) show that TPA can deliver one of the two signals needed for T cell activation and proliferation. Anti-T3 antibodies or mAb 9.3 can deliver the other signal. These antibodies, in collaboration with TPA, can induce IL-2 receptor expression and IL-2 secretion by T cells. These events precede T cell proliferation in a manner similar to antigen-induced T cell proliferation (1). Despite the similarity between the T3/Ti pathway and the 9.3 pathway, note that the T3/Ti complex can be
readily and completely modulated, while antigen 9.3 can only be partially modulated. The role of modulation of activation antigens in T cells needs to be determined more clearly.

mAb 9.3 was first reported in 1980 (15). In subsequent studies (17–21), it was established that this antibody identifies a major subpopulation of T cells. This population included T4⁺ helper/inducer T cells, and T8⁺ cytotoxic T cells. T8⁺ suppressor T cells do not react with mAb 9.3. Showing that mAb 9.3 is important in the mediation of T cell proliferation indicates that certain structures in a subpopulation of T cells are important in the activation process. Thus, multiple structures are involved in T cell activation. This is supported by the recent findings that several mAb against different antigens on murine T cells can augment or induce cloned T cell proliferation (22, 23). It is also possible that different structures are used by functionally distinct T cell populations. Relevant to this discussion is the recent finding by Bensussan et al. (24) that cloned T8⁺ suppressor T cells did not proliferate as a result of T3/Ti triggering. It would be of considerable interest to identify a molecule on this 9.3⁻ population that would mediate activation and proliferation of these T cells. Relevant to this discussion is the preliminary finding that 9.3⁻ T cells did not respond well to mitogens and anti-T3 mAb in the presence of TPA. These data suggest that activation mechanisms differ in different T cell subsets.

Our preliminary experiments indicate that mAb 9.3 augments anti-T3-induced T cell proliferation in the presence of TPA. This adds support to the thesis that the T3/Ti and 9.3 activation pathways are independent. Related to this point is the recent finding that mAb 9.3 can augment suboptimal doses of anti-T3 and various T cell mitogens to induce T cell proliferation. These results also underline the need to ascertain the relative importance of these activation pathways in normal T cell proliferation.

Summary

In previous studies (17–21), monoclonal antibody (mAb) 9.3 has been shown to react with a major population of human T cells, which include T4⁺ helper/inducer T cells and T8⁺ cytotoxic T cells. In this investigation, mAb 9.3 was shown to precipitate a disulfide-bonded dimer of a 44 kD polypeptide. Comodulation experiments showed that this molecule is not linked to T3/Ti or T11 antigens. mAb 9.3 was capable of inducing T cell proliferation in the presence of 12-0-tetradecanoyl phorbol-13-acetate (TPA). This effect was monocyte-independent. T cell activation with mAb 9.3 and TPA was associated with increases in interleukin 2 (IL-2) receptor expression and IL-2 secretion. mAb 9.3 did not activate T cells, even with the addition of IL-1 or IL-2. Modulation of the T3 complex did not abolish mAb 9.3-induced T cell proliferation in the presence of TPA. These results suggest that the 9.3 antigen may serve as a receptor for an activation pathway restricted to a T cell subset.

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