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An Efficient Th2-Type Memory Follows CD8+ Lymphocyte-Driven and Eosinophil-Mediated Rejection of a Spontaneous Mouse Mammary Adenocarcinoma Engineered to Release IL-4

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A retroviral infection was used to introduce the cDNA coding for mouse IL-4 into the parental cells of a spontaneous adenocarcinoma of BALB/c mice (TS/A-pc). Four clones releasing between 5 to 40 U of IL-4 (10^5 cells) in 48 h culture were selected. The secretion of IL-4 does not affect their in vitro growth, whereas their ability to form tumor in vivo inversely correlates with the amount of IL-4 secreted. Although morphologic observation suggested that the rejection of clone D5.40 cells (releasing 40 U of IL-4) depends on eosinophil cytolysis, lymphocyte depletion experiments showed that this required CD8+ lymphocyte guidance. Mice that had rejected D5.40 cells were immune to a subsequent challenge with TS/A-pc. This memory rests on the interaction between noncytotoxic lymphocytes, eosinophils, and IgG1 and IgE anti-TS/A Abs. Comparison of these memory mechanisms with those elicited by IL-2 gene-transduced TS/A cells shows that the kind of cytokine released by the tumor cells determines the type of response. This Th2 memory seems to be more efficient in protecting against a subsequent challenge of TS/A-pc than the Th1-type memory elicited by IL-2 gene-transduced TS/A cells. The Journal of Immunology, 1994, 153: 5659.

The immune scenario generated during tumor growth is progressively dominated by suppression (1, 2). As a result of its production of chemotactic and suppressor factors (3, 4), a tumor is infiltrated by leukocytes of various kinds whose functions are eventually inhibited (5). In its more advanced stages, this suppression expands and becomes systemic (1–2, 5).

In contrast, when a critical local concentration of cytokines able to induce a few immune mechanisms (inducer cytokines) is reached, either through their repeated injection at the tumor site, or when tumor cells are genetically engineered to produce cytokines (hereafter referred to as engineered cells), a rapid and potent inflammatory reaction is evident both in mice (6, 7) and in cancer patients (8). A major role in this reaction is often played by nonspecific leukocytes that massively infiltrate and kill tumor cells. It occasionally creates the setting for specific recognition of tumor-associated Ag and establishment of an immune memory (6, 7).

The features of the first inflammatory reaction against the same tumor and the effectiveness with which a memory is induced vary in the function of the inducer cytokine and its concentration (7–13). Because vaccination through engineered tumor cells is a new and attractive prospect (6, 7), we decided to explore the immunogenic features of tumor cells transduced with mouse IL-4 gene, and the mechanisms of rejection.
IL-4 is a typical Th2-derived cytokine that affects numerous aspects of the immune response (14), and in particular plays a major role in B and T cell growth (14), influences T cell differentiation by steering the balance toward Th2 (15), activates macrophages (14), promotes both IgG1 and IgE isotype switching (16, 17), controls the selective endothelium-driven transmigration of eosinophils (18), and enhances the cytolytic activity of effecter cells in Ab-dependent cellular cytotoxicity (ADCC) (19).

To get a realistic assessment of the immunogenic potential of IL-4-releasing tumor cells, we introduced the cDNA coding for murine IL-4 into the cells of a spontaneous, poorly immunogenic, mouse mammary adenocarcinoma. The in vivo growth of adenocarcinoma clones releasing 40 U IL-4 or less is inhibited by an eosinophil-dominated reaction that requires CD8⁺ lymphocyte guidance. Mice that have rejected IL-4-releasing adenocarcinoma cells are immune to a subsequent challenge with parental adenocarcinoma cells through a series of mechanisms typical of the immune responses reflected toward Th2 lymphocytes.

Materials and Methods

Mice

Normal 7-wk-old female BALB/cAnCr (BALB/c) (H-2b, Mlsb) mice (Charles River Laboratory, Calco, Italy) were treated in accordance with the European Community guidelines. When sublethal whole body irradiation was required, mice received 5 Gy from a ¹³⁷Cs source providing a dose rate of 0.5 Gy/min. Other groups of mice received 11 daily i.p. injections of 0.2 ml HBSS containing 300 μg of 11B11 anti-IL-4 mAb (17) or normal rat Ig starting 4 h before tumor challenge. Starting 1 h after challenge, other mice received 10 daily i.p. injections of 75 mg/kg of cyclosporin A (Sandimmun, Sandoz, Basel, Switzerland) diluted in 0.2 ml of HBSS. Two days before challenge, and 4, 3, days, 7 days, 10 days, and 13 days after challenge, other mice received six i.p. injections of 0.2 ml of HBSS containing a 1/20 dilution of anti-asialo GM1 rabbit anti-serum (Wako Chemicals GmbH, Dusseldorf, Germany), or 100 μg anti-CD4 (GK1.5 hybridoma, L3T4, American Type Culture Collection (ATCC), Rockville, MD), anti-CD8 (TIB-105 hybridoma, Lyt 2, ATCC), anti-granulocyte mAb (RB6-8C5 hybridoma; kindly provided by Dr. R. L. Coffman, DNAX Inc., Palo Alto, CA), or normal rat Ig purified from normal serum or ascitic fluid by passage through an anionic exchange column (DE 52, Whatman Ltd., Maidstone, United Kingdom) (10). Cytotoxicity analysis of the residual blood and spleen cells (Spc) from mice receiving these Ab showed that target leukocytes were selectively decreased to <1/5,000 blood leukocytes during treatment.

Tumors

TS/A is an highly aggressive and poorly immunogenic cell line established from the first in vivo transplant of a moderately differentiated mammary adenocarcinoma that spontaneously arose in a 20-mo-old multiparous BALB/c mouse (10-13). Several vials of the eighth in vitro passage of the TS/A parental cells (TSiA-pc) were stored in liquid nitrogen before thawing and culture by one or two passages in vitro in RPMI 1640 medium supplemented with 10% FCS, 50 μg/ml gentamicin, and 2 mM glutamine (all from Wittaker, Milan, Italy) (hereafter referred to as complete medium). TSiA-pc express class I but not class II H-2b alloantigens. Confluent monolayers (5 × 10⁶ cells/cm²) were treated with a 0.25% solution of trypsin (Sigma Chemical Co., St. Louis, MO) in HBSS, and used for in vitro and in vivo experiments. Mice were always challenged with 10⁶ TS/A cells, a dose 2.5 times higher than the minimal TS/A-pc 100% tumor-inducing dose in mice of the BALB/c strain (cnAnCr) of TS/A origin. A few groups of mice received injections of TS/A-pc pretreated with 60 μg/ml of mitomycin-C (Sigma Chemical Co.) 10³ cells/ml for 30 min at 37°C (Mit-C cells). A few mice were also challenged with 10³ cells from a clone of TS/A cells (B6.3600) transfected with the murine IL-2 gene and releasing 3600 units of IL-2 (10⁻⁶ cells/ml) in 48 h culture. B6.3600 cells induce a strong host inflammatory reaction that leads to their complete rejection (10). F1-F is a newborn BALB/c mouse-derived skin fibroblast line spontaneously transformed after the 15th in vitro passage. Its minimal 100% tumor-inducing dose is 10³ cells.

Retroviral infection

The L4SN and L4RevSN vectors were constructed by introducing the murine IL-4 cDNA into the Hpal site of the LXSΝ vector (20). The IL-4 cDNA was the 480-bp BamHI-ScaI fragment contained in the LXSΝ and L4RevSN vectors. The titer was evaluated by HT-2 cell [³H]TdR uptake assay at weekly intervals. The IL-4 gene-transduced clones were denoted by the mean titer of the IL-4 released.
plasmid pD2-E3 (ATCC, no. 37561). This fragment was blunt-ended by Klenow polymerase-mediated fill-in. The IL-4 cDNA was oriented by digestion with XmnI, which cuts at position 430 of the IL-4 sequence. Vector DNAs were converted to the corresponding virus by transfection into the ß2 eotropic packaging cell line (21). Transfected cells were selected with 0.8 mg/ml of G418 (Life Technologies, Inc. Grand Island, NY) and then used to generate supernatant containing virus at a titer of \(10^7 \text{ cfu/ml}\). TS/A cells were infected for 2 h with 5 ml of this supernatant in the presence of 8 mg/ml of polybrene, grown for an additional 24 h, and then selected in the presence of 1.2 mg/ml of G418. Resistant cells were cloned by limiting dilution in selective medium, and the single cell clones were expanded and subcloned in this medium.

**IL-4 titration**

Four representative IL-4 secreting clones, D1.5, D2.5, D4.20, and D5.40 were used. The last number of their denomination indicates the mean units of IL-4 released by \(10^5\) cells of each clone cultured for 48 h in 1 ml of complete medium, evaluated as the ability to support the growth of the mouse line HT-2, which is both IL-2- and IL-4-responsive (22). The titer that gave 50% of the maximal [H]Tdr uptake was determined by a probit analysis computer program and calibrated against the biologic reference reagent murine IL-4 (12).

**In vivo evaluation of tumor growth**

BALB/c mice were challenged s.c. in the middle of the left (primary challenge) or right (secondary challenge) flank with 0.2 ml of a single cell suspension containing the indicated number of tumor cells. The cages were coded, and the incidence and growth of tumors were evaluated twice weekly in a fashion blind to the group in which they had been treated. Neoplastic masses were measured with calipers in the two perpendicular diameters for 60 days. Mice tumor-free at the end of this period were classed as survivors. No de novo tumor incidence was observed 30 days after challenge. Latency and survival times were considered as the periods (in days) respectively between challenge and the growth of a neoplastic mass >3 and >10 mm mean diameter. Only mice that eventually developed tumors were considered. In control mice, all parental TS/A and F1-T tumors that overcame the latency threshold grew progressively, and no spontaneous regressions were observed. All mice bearing neoplastic masses of >10 mm mean diameter were killed for humane reasons.

**Morphologic analysis**

For histologic evaluation, tissue samples from groups of four mice killed 3, 5, and 10 days after tumor challenge were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 \(\mu\)m, and stained with hematoxylin-eosin. Neutrophils were characterized by segmented or doughnut nuclei, and were clearly distinguishable from eosinophils by the absence of eosinophilic granules. For electron microscopy, specimens were fixed in cacodylate-buffered 2.5% glutaraldehyde, postfixed in osmium tetroxide, and then embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate-lead citrate. In addition to many ultrastructural differences, the typical oval granule with the central rectangular crystalloid in the cytoplasm of the eosinophils enabled them to be unmistakably distinguished from neutrophils (13).

**Cellular cytotoxicity**

CTLs were generated by culturing \(10^7\) responder Spc and \(5 \times 10^5\) Mit-C stimulator tumor cells for 6 days. Cytotoxicity was assayed in a 48-h [H]Tdr release assay (23) by mixing various concentrations of effector cells with \(5 \times 10^3\)-labeled target cells at 50:1, 25:1, 12:1, and 6:1 E:T ratios in round-bottom 96-well microtiter plates in triplicate. Lysis was determined as previously described in detail (10), and values were expressed as LUs/10^5 effector cells calculated according to the equation of Pross (24).

**Immune sera**

Groups of 12 mice were challenged once or six times twice a week in the left flank with 0.2 ml of a single cell suspension containing 10^5 trypan blue dye excluding D5.40 clone cells or Mit-C TS/A-pc. Sera were collected 7 days after the last injection. Pooled sera from four mice were referred to as D5.40 or Mit-C TS/A-pc immune 1× and immune 6× sera. Normal sera were pools from four age-matched, untreated mice.

**ADCC**

Target \(5 \times 10^5\) [H]Tdr-labeled TS/A-pc were added to flat-bottom wells of microtiter plates, incubated for 2 h at 4°C with a 1:10 dilution in HBSS of normal or immune sera, and washed with HBSS. Effector Spc from untreated mice and mice challenged 7 days before with Mit-C TS/A-pc or D5.40 cells were then added at 50:1, 25:1, and 12:1 E:T ratios to each well in triplicate, and lysis was determined as described.

**Serum concentration of Ig isotypes**

The concentration of IgG1, IgG2a, IgG2b, IgM, IgA, and IgM isotypes in pools of normal and immune sera was determined by the radial immunodiffusion test (The Binding Site, Birmingham, UK). Selective depletion of IgG1 and IgE was obtained by incubating the immune serum overnight at 4°C on a rotating platform with agarose-antimouse IgG1 (Sigma Chemical Co., St. Louis, MO) and sheep antimouse IgE Ab (The Binding Site) covalently linked to CNBr-activated Sepharose CL-4B (Pharmacia, Colombo Monzese, Italy). The adsorbed serum contained neither detectable IgG1 as shown by radial immunodiffusion, nor anti-TS/A IgE as shown by cytometry, whereas the concentration of the other Ig isotypes was not appreciably modified compared with immune serum.

**Flow cytometry**

TS/A-pc from in vitro cultures were washed twice with cold HBSS supplemented with 2.0% BSA and 0.05% sodium azide, and stained with a 1:10 dilution in HBSS-azide-BSA of normal or immune sera. The following mAb were used as second step Ab: FITC F(ab')2 goat anti-mouse IgG1 (Technogenetics, Milan, Italy); FITC rati anti-mouse IgG2a, IgG2b, IgG3, IgA, IgM, and biotin-conjugated rat anti-mouse Ig E (PharMingen, San Diego, CA). Normal mouse Ig (to block residual binding sites on the anti-mouse Ig) and streptavidin-phycocrytin (Technogenetics) were also added. Spc from normal or immune mice, and nylon wool column effluent Spc (>90% Thy 1.2+, hereafter referred to as T-Spc) (11) were directly incubated with these mAb or with anti-Thy 1.2 mAb (DuPont NEN, Boston, MA). All labeling steps were followed by incubation for 30 min at 4°C, and separated by two washes with cold HBSS-azide-BSA. Stained cells were analyzed with a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). Dead cells were gated on the basis of forward and sideways side scatter. In each experiment, 10^6 viable cells were analyzed.

**Adoptive transfer**

Normal mice were sublethally irradiated, and 72 h later injected i.v. with 10^7 T-Spc from untreated mice and mice that had rejected D5.40 cells 30 days earlier. After 96 h, all mice were challenged in the left flank with 10^5 TS/A-pc. At 24 and 72 h after challenge, a few received an i.v. administration of 0.2 ml of a pool of normal or D5.40-immune 1× serum.

**Lymphokine production**

In one set of experiments, 10^7 responder inguinal lymph node cells from mice primed with Mit-C TS/A-pc, D5.40, and B6.3600 cells 10 days earlier were cultured with 10^5 stimulator Mit-C TS/A-pc for 6 days. Two \(\times 10^5\) of recovered lymphocytes were added to the wells of 96-well flat-bottom plates (Nunc, Roskilde, Denmark) precoated with 1 \(\mu\)g/well of anti-CD3 mAb (PharMingen), and cultured for an additional 18 h at 37°C in a humidified 5% CO, atmosphere. In another set of experiments, mice challenged with Mit-C TS/A-pc, D5.40, and B6.3600 cells were rechallenged with TS/A-pc. At 4, 24, and 48 h after challenge mice were killed and inguinal lymph node cells were stimulated with anti-CD3 mAb as specified above. The IL-4 and IFN-γ titer in these supernatants was evaluated by ELISA (PharMingen).
Table 1. Oncogenicity in syngeneic BALB/c mice of IL-4-secreting tumor cells

<table>
<thead>
<tr>
<th>Challenging Tumor Cells*</th>
<th>Tumor Takes/Total Mice</th>
<th>Latency (Time /days)</th>
<th>Survival (Time /days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS/A-pc</td>
<td>15/15 (100%)</td>
<td>11 ± 1</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>NEO.i</td>
<td>15/15 (100%)</td>
<td>10 ± 3</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>D.rev</td>
<td>15/15 (100%)</td>
<td>12 ± 1</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>D2.5</td>
<td>12/15 (80%)</td>
<td>26 ± 5</td>
<td>39 ± 4</td>
</tr>
<tr>
<td>D5.40</td>
<td>11/15 (73%)</td>
<td>25 ± 3</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>D4.20</td>
<td>8/15 (53%)</td>
<td>24 ± 1</td>
<td>46 ± 2</td>
</tr>
<tr>
<td>D5.40</td>
<td>12/60 (20%)</td>
<td>28 ± 2</td>
<td>47 ± 7</td>
</tr>
</tbody>
</table>

* Mice were challenged with 10⁸ cells.

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Statistical analysis

All in vitro experiments were performed in triplicate, and repeated independently at least three times. A representative experiment is shown. All in vivo experiments were performed three or more times with groups of 4 to 6 mice, and were cumulated as they gave homogeneous results. The significance of differences in tumor takes was determined by Fisher’s exact method, whereas those in latency and survival time and in vitro data were determined by a two-tailed Wilcoxon signed test.

Results

IL-4 gene transfer into TS/A cells

TS/A-pc were infected with supernatant containing a virus from the ψ2 packaging cell line originally transfectected with L4SN and L4RevSN vectors containing the murine IL-4 cDNA in right and reverse orientation, respectively (21), under the long terminal repeat promoter, and the neomycin resistance gene under the SV4 promoter (Fig. 1, upper panel). Neomycin-resistant colonies were individually collected, cloned for limiting dilution and, after analysis of IL-4 expression by reverse transcriptase-PCR (not shown), tested for resistance gene under the SV4 promoter (Fig. 1, lower panel). Neomycin-resistant colonies were individually collected, cloned for limiting dilution and, after analysis of IL-4 expression by reverse transcriptase-PCR (not shown), tested for resistance gene under the SV4 promoter (Fig. 1, upper panel). Neomycin-resistant colonies were individually collected, cloned for limiting dilution and, after analysis of IL-4 expression by reverse transcriptase-PCR (not shown), tested for resistance gene under the SV4 promoter (Fig. 1, upper panel).

To determine whether the local presence of IL-4 was indeed responsible for the antitumor reaction, starting 4 h before challenge, mice received 11 i.p. daily injections of 300 µg of normal rat Ig or neutralizing anti-IL-4 mAb (17). D5.40 cells were again rejected by 80% of mice receiving normal rat Ig, whereas in mice receiving anti-IL-4 mAb they always gave rise to tumors that went past the

Oncogenicity of IL-4-secreting cells

To determine whether constitutive secretion of IL-4 affects the ability to form tumor, mice were challenged with a dose 2.5 times higher than the minimal 100% tumor-inducing dose of TS/A-pc. All mice challenged with TS/A-pc, D.rev, and NEO.i cells developed tumor with a similar progression pattern. By contrast, only 80%, 73%, 53%, and 20% of mice challenged with D2.5, D1.5, D4.20, and D5.40 cells, respectively, displayed tumors. These were characterized by delayed growth patterns (Table I), suggesting that the oncogenicity of IL-4-engineered cells inversely correlates with the amount of IL-4 secreted.

To determine whether the local presence of IL-4 was indeed responsible for the antitumor reaction, starting 4 h before challenge, mice received 11 i.p. daily injections of 300 µg of normal rat Ig or neutralizing anti-IL-4 mAb (17). D5.40 cells were again rejected by 80% of mice receiving normal rat Ig, whereas in mice receiving anti-IL-4 mAb they always gave rise to tumors that went past the

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**FIGURE 1.** Growth of 10⁸ D5.40 cells in mice that received 11 daily i.p. injections of 300 µg of normal rat Ig or neutralizing anti-IL-4 mAb (11B11) starting 4 h before challenge. Each line refers to a single mouse. Arrows: days of Ig injection.
latency threshold. Interestingly, 8 days after the last injection of anti-IL-4 mAb, one tumor started to regress and eventually was completely rejected (Fig. 2).

Morphologic features of D5.40 cell rejection

To get an insight into the mechanisms associated with the rejection of engineered cells, mice challenged with TS/A-pc, D.rev, NEO.i, and D5.40 cells were killed 3, 5, and 10 days after challenge, and the tumor growth area and tumor-draining inguinal lymph nodes were processed for light and electron microscopy. TS/A-pc, D.rev, and NEO.i cells similarly gave rise to poorly infiltrated tumors with many mitotic figures invading the fibroadipose tissue and epidermis (not shown). By contrast, marked eosinophil and macrophage infiltration and many eosinophils adhering to or crossing the walls of tumor vessels were evident 3 days after challenge (Fig. 3). At day 5, neoplastic cells in various stages of degeneration surrounded by a massive reactive infiltrate composed of eosinophils, macrophages, and some lymphocytes, and a large necrotic area, were evident. At the borders of the necrotic area, numerous neutrophils had now replaced eosinophils and macrophages. At day 10, D5.40 cells were replaced by granulocyte tissue (not shown). In contrast, in mice receiving anti-IL-4 mAb, all tissue sections displayed almost exclusively viable tumor cells in active proliferation and a little peripheral reactive infiltrate only, as in mice challenged with TS/A-pc, D.rev, and NEO.i cells (data not shown).

Tumor-draining lymph nodes of mice challenged with D5.40 enlarged progressively up to day 10. On day 3, the paracortical area only was hyperplastic, whereas on day 5, both cortical and paracortical areas were expanded, follicles were evident, and germinal centers showed activated B cells, tingible-body macrophages, and eosinophils (Fig. 4). The hyperplastic paracortical area was characterized by an increased number of small lymphocytes, immunoblasts, macrophages, and eosinophils. The presence of eosinophils intermingled with centroblast or centrocyte clusters was also a common feature in the follicular germinal centers. The medulla showed cords packed with plasma cells. Leukocyte traffic was evident across the epithelioid venules. In mice challenged with TS/A-pc, D.rev, and NEO.i cells, all these features were much less pronounced or absent (data not shown). No metastatic TS/A cells were found in any lymph nodes.

The role of host reactivity in D5.40 cell rejection

These findings suggest that D5.40 cells are potentially oncogenic, but are promptly rejected because of the IL-4-elicited host reaction, which is mostly associated with eosinophils that extravasate from near vessels and kill D5.40 cells. Resistance to these cells was abolished by sublethal irradiation (Table II), showing that radiosensitive mechanisms of host reactivity play a crucial role. It was also abolished by cyclosporin A, suggesting that active release of additional cytokines is an important feature. Because of the quick reversibility of cyclosporin A action, one-third of the tumors progressively growing during cyclosporin A treatment regressed when it was interrupted. Resistance was not affected by the removal of asialo GM1+ cells, whereas it was abolished by anti-granulocyte mAb, confirming the major role of granulocytes in killing D5.40 cells. Depletion of CD4+ T cells did not significantly impair this resistance, but it was abolished by CD8+ T cell depletion. An interaction between eosinophils and CD8+ T cells was shown by the histologic finding that D5.40 tumors growing in mice treated with anti-CD8 mAb were infiltrated by very few eosinophils and other reactive cells, as in TS/A-pc or NEO.i tumors. Moreover, even D5.40 cells near reactive leukocytes did not display signs of damage (Fig. 5). A similarly sparse infiltrate and signs of cell death were found in D5.40 tumors growing in mice immunosuppressed by cyclosporin A or anti-granulocyte serum (data not shown).

Inability of D5.40 cells to elicit CTL

Despite this strong and efficient reaction, no cytotoxicity to TS/A-pc was found in Spc from mice that had rejected D5.40 cells 1 mo before (data not shown), nor from those that were tumor-free 1 wk after a single or six twice-weekly D5.40 challenges (Table III). Similar injections of Mit-C TS/A-pc elicited a detectable CTL response. Moreover, in vitro restimulation with D5.40 cells of Spc from mice challenged one or six times with either TS/A-pc or D5.40 did not elicit a significant response to TS/A-pc, whereas a CD8+ cell-mediated and TS/A-pc-restricted cytotoxicity was stimulated by TS/A-pc.

Serum Ig isotype switch

Because IL-4 is also an Ig isotype switch factor (16, 17), the influence of IL-4 released by D5.40 cells on serum Ig isotype was investigated. A significant increase in IgG1 and IgA, and a decrease in IgG2a isotype, were detected in D5.40 immune 6 × sera (Table IV). Because this increase cannot be detected in D5.40 immune 1 × sera, it presumably requires protracted stimulation. Alternatively, these initially produced Ig are adsorbed by the growing D5.40 cells. Moreover, in the spleen from D5.40-challenged mice, flow cytometry showed an increase in Spc-expressing membrane IgE (Table V). No evidence for Ig isotype switch or increased surface IgE+ cells was found in the sera and spleens from mice that received Mit-C TS/A-pc injections.

The role of anti-TS/A Ab

Morphologic findings displaying a massive plasma cell reaction in tumor-draining lymph nodes during the rejection of D5.40 cells also pointed to an Ab response to TS/A-pc. In effect, when the ability of D5.40 immune 1× or 6× sera to stain TS/A-pc was evaluated by flow cytometry, 10%
FIGURE 4. Tumor-draining inguinal lymph node 5 days after D5.40 challenge. Panel a: an enlarged follicle and a germinal center (arrowheads) with activated B lymphocytes in all forms of transition from centroblasts to centrocytes. Several tingible-body macrophages (arrows) containing remnants of cell nuclei and eosinophils are also evident (original magnification X400). Panel b: leukocyte traffic with numerous eosinophils passing through the epithelioid venule (arrowheads) is evident in the hyperplastic paracortical area. Panel c: medullary cords packed with plasma cells (arrowheads) and lymphocytes with lymphoplasma cytoid features. Panels’ original magnification: a, X400; b, X630; c, X1000.

and 25% of TS/A-pc cells, respectively, were highly positive (Table VI). In immune 6X sera, the staining was mostly observed for IgG1, IgA, and IgE subclasses. Moreover, sera from mice that rejected a D5.40 cell challenge mediated ADCC against TS/A-pc. The size and cell composition of the spleen from mice that rejected
D5.40 cells were not significantly different from those of normal mice and Mit-C TS/A immune mice (data not shown). The stronger ADCC displayed by these Spc than by Spc from normal (Fig. 6) or Mit-C TS/A immune (data not shown) mice suggests enhancement of the cytotoxic capacity of ADCC effector cells by the IL-4 (19) released by D5.40 cells.

**In vivo memory to TS/A-pc**

To test whether the rejection of IL-4-producing cells leads to an immune memory to TS/A-pc, mice with no tumors after the challenge with D1.5, D2.5, D4.20, and D5.40 cells performed 30 days earlier in the left flank were challenged with TS/A-pc or F1-F in the right flank. Irrespective of the amount of IL-4 secreted by the clone cells used in the first challenge, all mice displayed total protection against the subsequent TS/A-pc challenge and no protection against F1-F challenge (Table VII).

To evaluate a few requirements for the induction of this memory, groups of mice were immunosuppressed during the first challenge with D5.40 cells. D5.40 tumor-free mice after the interruption of cyclosporin A treatment (Table II) were fully resistant to the subsequent TS/A-pc challenge (Table VII). Immunosuppression with anti-CD4, which slightly impaired the first D5.40 cell rejection (Table II), significantly affected the ability to acquire an immune memory to TS/A-pc (Table VII).

Surprisingly, when the features of this secondary rejection of TS/A-pc were studied morphologically, the tumor-reactive infiltrate was again predominantly composed of eosinophils and macrophages, with relatively few lymphocytes (Fig. 7). Selective depletion of granulocytes decreased the ability to reject TS/A-pc to 50%. A smaller decrease followed depletion of CD4⁺ T cells, whereas no resistance was left when CD8⁺ T cells were removed (Table VIII).

To further study this memory rejection pattern, immune serum and T-Spc from mice that had rejected D5.40 cells 30 days earlier were adoptively transferred to sublethally irradiated mice. Transfer of immune serum gave about 40% protection. Immune T-Spc protected fewer mice. Transfer of both immune serum and T-Spc afforded full protection. IgG1 and IgE Ab seem to play a major role, because protection was no longer afforded by immune serum from which they had been selectively depleted (Table IX).

**Imbalance of IL-4 and IFN-γ production in mice primed with D5.40 cells**

As the presence of distinct cytokines at the Ag priming determines the differentiation into Th1 or Th2 lymphocytes (25), we compared the effect of B6.3600 and D5.40 priming on IFN-γ and IL-4 production by tumor-draining lymph node cells upon restimulation. At 10 days after B6.3600, D5.40, or Mit-C TS/A-pc challenge, mice were killed, and lymphocytes from inguinal lymph nodes draining the challenge area were cultured for 6 days with Mit-C TS/A-pc, and then further stimulated with anti-CD3 mAb for 18 h. In vivo priming with B6.3600 induced a substantial IFN-γ release and a moderate release of IL-4 during the last 18-h stimulation (Fig. 8, left panel). Almost the same level of IL-4 was released by lymph node cells from mice primed with D5.40 cells, whereas IFN-γ release was 10 times lower, displaying a value similar to that of Mit-C TS/A-pc-primed mice.

In another set of experiments, 10 days after challenge with B6.3600, D5.40, or Mit-C TS/A-pc, mice without palpable tumors were rechallenged with TS/A-pc and killed 4, 24, and 48 h afterward. Lymphocytes from inguinal lymph nodes draining the rechallenge area were then stimulated with anti-CD3 mAb for 18 h. The IL-4 that was released was always below the detection limit. The amount of IFN-γ released by mice primed with B6.3600 and D5.40 cells progressively increased and decreased respectively during the 48 h of rechallenge, clearly showing the importance of priming on the acquisition of a distinct lymphokine-producing phenotype (Fig. 8, right panel).

**Discussion**

The data reported here show that an otherwise lethal challenge of TS/A tumor is rejected, and induces memory Ig and T lymphocytes, as the outcome of the IL-4 released. These findings are strengthened by the consideration that they are obtained with an aggressive and poorly immunogenic adenocarcinoma, as most spontaneously arising tumors are. No immunity nor reactive T lymphocytes are elicited by a similar injection of Mit-C TS/A-pc alone or mixed with conventional adjuvants (10–12, 39).

The efficiency with which the inflammatory reaction first elicited by engineered TS/A cells overcomes their oncogenic capacity is directly proportional to the amount of IL-4 they secrete. Previous data obtained with TS/A cells engineered to release IL-2 have shown that when a strong
inflammatory reaction leads to the quick disappearance of the incipient tumor, the Ag load is not sufficient to trigger the immune system efficaciously. A less prompt reaction allows an initial growth and the formation of a larger tumor mass. When it is eventually rejected, massive amounts of tumor cell debris are evident in the rejection area, while tingible-body macrophages and granulocytes migrate from it to the draining lymph node, where activation of T cell areas becomes evident (7, 10).

The relatively slow inflammatory reaction elicited by D5.40 cells is morphologically characterized by an early influx of eosinophils that enter the proliferating tumor mass and kill tumor cells. Both findings resemble those described by Tepper, Pattengale, and Leder (26); Tepper, Coffman, and Leder (27); and Golumbek et al. (28) for several tumors engineered to release high amounts of IL-4. However, as the TS/A clones used in this study display a single retroviral integration (data not shown) and release 5

**FIGURE 5.** Histologic and ultrastructural features of D5.40 cells growing in mice treated with anti-CD8 mAb. At 5 days after challenge very few reactive cells are present in both the central (panel a) and peripheral (panel b) tumor areas. Electron micrographs show that tumor cells (T) near or in contact with eosinophils (E) (panel c), neutrophils (N), or macrophages (M) (panel d) are free from evident damage. Panels' original magnification: a, b, ×630; c, d, ×4700.
to 40 units of IL-4 only, mechanisms difficult to see or not triggered when a very strong inflammatory reaction is triggered by high levels of IL-4 (26-28) are revealed by our D5.40 cells. However, they also made it clear that CD8+ lymphocytes are essential, whereas CD4+ T cells are less important. These tumor-infiltrating lymphocytes are too few to directly contribute to tumor rejection. Rather, low IL-4 concentrations may make them of prime importance in promoting eosinophil activity through the release of cytokines, whose secretion is inhibited by cyclosporin A. In mice only (13). In this setting, CD4+ lymphocyte depletion sufficed to block TS/A rejection (12). The influence of CD8+ lymphocytes was not investigated.

The features of the reaction toward D5.40 cells are distinctly different from those associated with the rejection of IL-2 gene and IFN-γ gene-engineered TS/A-pc, namely dominance of neutrophils (10) and dominance of macrophages (11), respectively. Therefore, the repertoire of leukocytes recruited and the efficacy of the reaction directly depend on the lymphokine secreted by the engineered tumor cells.

Table III. Generation of TS/A-specific CTL in Spc from normal mice and mice injected with Mit-C TS/A-pc or D5.40 cells

| Responder Lymphocytes from Mice Challenged with:* | Stimulator Cells* | Cytotoxicity to:  
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TS/A-pc</td>
</tr>
<tr>
<td>Nothing</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>Mit-C TS/A-pc</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D5.40</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* 10⁶ cells were injected once or six times twice a week in the right flank. Mice were killed 7 days after last challenge.

* Cytotoxicity was evaluated in a 48-h [3H]Tdr release assay and expressed as L50/10¹ × 10⁶.

* Responder Spc were cultured for 6 days with Mit-C stimulator cells at 20:1 responder to stimulator ratio.

* Mice without palpable tumors 1 wk after last challenge.

* Cytotoxicity disappeared after treatment of the effector cells with either anti-Thy and anti-CD8 mAb and C, whereas it was not affected by similar treatment with anti-CD4 mAb.

### Table IV. Levels of serum Ig isotypes in pools of sera from normal mice and mice injected with Mit-C TS/A-pc or D5.40 cells

<table>
<thead>
<tr>
<th>Sera from Mice Challenged with:*</th>
<th>lgG1</th>
<th>lgG2a</th>
<th>lgG2b</th>
<th>lgG3</th>
<th>lgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>0.26±0.05</td>
<td>2.6±0.02</td>
<td>0.17±0.05</td>
<td>0.17±0.02</td>
<td>0.75±0.04</td>
<td>0.30±0.06</td>
</tr>
<tr>
<td>Mit-C TS/A-pc</td>
<td>0.27±0.07</td>
<td>2.5±0.02</td>
<td>0.16±0.04</td>
<td>0.17±0.03</td>
<td>0.73±0.03</td>
<td>0.31±0.03</td>
</tr>
<tr>
<td>6</td>
<td>0.28±0.09</td>
<td>2.9±0.03</td>
<td>0.17±0.06</td>
<td>0.18±0.04</td>
<td>0.75±0.03</td>
<td>0.33±0.02</td>
</tr>
<tr>
<td>D5.40</td>
<td>0.20±0.03</td>
<td>1.2±0.04*</td>
<td>0.16±0.06</td>
<td>0.13±0.05</td>
<td>0.64±0.08</td>
<td>0.23±0.08</td>
</tr>
<tr>
<td>6</td>
<td>0.73±0.11*</td>
<td>1.7±0.03*</td>
<td>0.20±0.04</td>
<td>0.16±0.05</td>
<td>1.00±0.06*</td>
<td>0.31±0.04</td>
</tr>
</tbody>
</table>

* As in Table III.

* Values are the arithmetical mean ± SD of three sera pools from four mice.

* As in Table III.

* Value significantly different (p < 0.05) from both those of untreated mice and mice challenged with Mit-C TS/A-pc.

### Table V. Surface IgE-positive cells in spleens from normal mice and mice injected with Mit-C TS/A-pc or D5.40 cells

| Spc from Mice Challenged with:* | Cells | Times | % of IgE* Spc
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>—</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>Mit-C TS/A-pc</td>
<td>1</td>
<td>6</td>
<td>5.3</td>
</tr>
<tr>
<td>D5.40*</td>
<td>1</td>
<td>6</td>
<td>8.6</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>13.8</td>
<td></td>
</tr>
</tbody>
</table>

* As in Table III.

* Detected by flow cytometry using a monoclonal biotin-conjugated rat anti-mouse IgE followed by streptavidin-phycoerythrin. Control values did not exceed 2.0%.

### Table VI. Levels of serum Ig isotypes in pools of sera from normal mice and mice injected with Mit-C TS/A-pc or D5.40 cells

<table>
<thead>
<tr>
<th>Sera from Mice Challenged with:*</th>
<th>lgG1</th>
<th>lgG2a</th>
<th>lgG2b</th>
<th>lgG3</th>
<th>lgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>0.26±0.05</td>
<td>2.6±0.02</td>
<td>0.17±0.05</td>
<td>0.17±0.02</td>
<td>0.75±0.04</td>
<td>0.30±0.06</td>
</tr>
<tr>
<td>Mit-C TS/A-pc</td>
<td>0.27±0.07</td>
<td>2.5±0.02</td>
<td>0.16±0.04</td>
<td>0.17±0.03</td>
<td>0.73±0.03</td>
<td>0.31±0.03</td>
</tr>
<tr>
<td>6</td>
<td>0.28±0.09</td>
<td>2.9±0.03</td>
<td>0.17±0.06</td>
<td>0.18±0.04</td>
<td>0.75±0.03</td>
<td>0.33±0.02</td>
</tr>
<tr>
<td>D5.40*</td>
<td>0.20±0.03</td>
<td>1.2±0.04*</td>
<td>0.16±0.06</td>
<td>0.13±0.05</td>
<td>0.64±0.08</td>
<td>0.23±0.08</td>
</tr>
<tr>
<td>6</td>
<td>0.73±0.11*</td>
<td>1.7±0.03*</td>
<td>0.20±0.04</td>
<td>0.16±0.05</td>
<td>1.00±0.06*</td>
<td>0.31±0.04</td>
</tr>
</tbody>
</table>

* As in Table III.

* Values are the arithmetical mean ± SD of three sera pools from four mice.

* As in Table III.

* Value significantly different (p < 0.05) from both those of untreated mice and mice challenged with Mit-C TS/A-pc.
Table VI. Ab against TS/A-pc cells in sera from normal mice and mice injected with Mit-C TS/A-pc or D5.40 cells

<table>
<thead>
<tr>
<th>Sera from Mice Challenged with</th>
<th>% of TS/A-pc Stained by mAb to Anti-mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ig</td>
</tr>
<tr>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Mit-C TS/A-pc</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>D5.40c</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>6</td>
</tr>
</tbody>
</table>

* As in Table III.

* Evaluated by flow cytometry. Control values in the absence of mouse serum never exceeded 2.0%.

* As in Table III.

FIGURE 6. ADCC of Spc from untreated mice (panel A) and mice challenged once with D5.40 cells (panel B) in the presence of normal or D5.40 immune 1× serum. Spc and sera were collected 7 days after D5.40 challenge. Data are expressed as percentages of [3H]TdR release with LU_{107} Spc in brackets.

Table VII. Acquisition of an immune memory to TS/A-pc after the rejection of IL-4-releasing TS/A clones

<table>
<thead>
<tr>
<th>Challenging cells</th>
<th>Immunosuppressive treatment</th>
<th>TS/A-pc</th>
<th>F1:F</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>0/15 (0%)</td>
<td>0/12 (0%)</td>
</tr>
<tr>
<td>Mit-C TS/A-pc</td>
<td></td>
<td>0/15 (0%)</td>
<td>0/12 (0%)</td>
</tr>
<tr>
<td>D5.40</td>
<td>anti-CD4</td>
<td>6/6d (100%)</td>
<td>ND</td>
</tr>
<tr>
<td>D5.40</td>
<td>Cyclosporin A</td>
<td>6/6d (100%)</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Values significantly different (p < 0.001) from those observed in mice injected with Mit/C TS/A-pc.

* Not done.

In contrast with the nonspecific nature of this reaction, all mice that rejected IL-4-releasing cells are fully protected against a subsequent TS/A-pc challenge, even more effectively than those rejecting TS/A cells transfected with IL-2 gene (11). Inguinal lymph nodes draining the area of D5.40-challenged mice enlarge progressively during the first 10 days, displaying the arrival of tingible-body macrophages and eosinophils that may act as tumor APCs (7, 31), and evident lymphocyte activation. Depletion data underscored a marginal role for CD4+ lymphocytes in memory induction and a central role of both CD4+ and CD8+ lymphocytes in secondary TS/A-pc rejection. A similar T lymphocyte-dependent memory has been observed after rejection of RENCA cells engineered to release IL-4 (28). However, unexpectedly for a memory reaction, eosinophils, too, seemed to play a critical role. They are the predominant reactive cells at the secondary TS/A-pc rejection sites and their depletion markedly affects the efficacy of the reaction.

On the other hand, a marked humoral response accompanies the B lymphocyte and plasma cell activation observed in the draining lymph node. After repeated D5.40 challenges, the levels of serum IgG1 and IgA, and the number of IgE+ Spc, increase, pointing to an isotype-switching role of the IL-4 secreted by D5.40 cells, alone and along with other secondary cytokines secreted by activated T cells, as could be expected in Th2-deflected immune responses (32). Moreover, IgG1 and IgE bound to TS/A-pc may mediate ADCC, suggesting a major role for the humoral response. Both isotype switch and anti-TS/A
FIGURE 7. Histologic and ultrastructural features of the cell reaction to TS/A-pc cells injected s.c. in the right flank of mice that have rejected a D5.40 cells challenge performed 30 days earlier in the left flank. At 3 days after TS/A-pc challenge, a marked cellular infiltrate constituted by eosinophils, macrophages, and lymphocytes is evident not only in the periphery of the tumor (panel a) but also around and between central neoplastic cell clusters (panel b). Ultrastructurally, neoplastic cells in contact with eosinophils, macrophages, and lymphocytes display evident signs of damage (panels c and d). Eosinophils and macrophages are the predominant reactive cell population at the edges of the initial necrotic areas (panel d). E: eosinophils; L, lymphocytes; M: macrophages; T: TS/a-pc. Panels' original magnification: a, b, ×630; c, ×2750; d, ×4900.

Ig production are characteristic of the memory elicited by D5.40 cells, because they are not found in mice challenged with Mit-C TS/A-pc, or with IL-2 or IFN-γ gene-transfected TS/A-pc cells (Modesti, manuscript in preparation). Adoptive transfer experiments show that only partial protection against a subsequent TS/A-pc challenge could be transferred by either D5.40 immune sera or T-Spc. The full protection stemming from their combination points to a major interaction between humoral and cellular immunity. In addition, selective depletion of IgG1 and IgE markedly reduce the ability of D5.40 immune serum to transfer protection to TS/A-pc, suggesting that it is indeed
Th2-dependent Ab isotypes that play a major role. No protection was evident against a F1-F tumor challenge, showing the selectivity of these memory mechanisms.

These multiple facets of the D5.40 cell-elicited memory make it difficult to tease apart the interactions between the various reaction mechanisms. In particular, CD8⁺ lymphocytes, in sharp contrast with the evidence of their central role in the D5.40 cell-induced memory, do not display any cytolytic activity to TS/A-pc either during or after D5.40 cell rejection, nor after restimulation in vitro with D5.40 cells. This inability to stimulate CD8⁺ lymphocytes to non-cytolytic cells (33, 34), trigger what is normally considered the Th2 pattern of cytokines in both CD8⁺ and CD4⁺ lymphocytes, and thus guide eosinophil, macrophage, and B cell activation. In naive conditions, this Th2 response is triggered by IL-4 in CD8⁺ lymphocytes only, whereas after D5.40 rejection, expanded CD8⁺ and CD4⁺ lymphocytes release Th2 cytokines after TS/A-pc recognition.

In D5.40-immune mice, eosinophils and other effector leukocytes are further guided by anti-TS/A-pc Ig. Interestingly, ADCC effectors from D5.40-immune mice are local IL-4 may switch CD8⁺ lymphocytes to non-cytolytic cells (33, 34), trigger what is normally considered the Th2 pattern of cytokines in both CD8⁺ and CD4⁺ lymphocytes, and thus guide eosinophil, macrophage, and B cell activation. In naive conditions, this Th2 response is triggered by IL-4 in CD8⁺ lymphocytes only, whereas after D5.40 rejection, expanded CD8⁺ and CD4⁺ lymphocytes release Th2 cytokines after TS/A-pc recognition.

In D5.40-immune mice, eosinophils and other effector leukocytes are further guided by anti-TS/A-pc Ig. Interestingly, ADCC effectors from D5.40-immune mice are

---

**Table VIII.** Leukocytes responsible for the immune memory to TS/A-pc acquired after the rejection of D5.40 cells

<table>
<thead>
<tr>
<th>First Challenge with D5.40</th>
<th>Second Challenge with TS/A-pc</th>
<th>Immunosuppressive treatment during the TS/A-pc challenge</th>
<th>Surviving/total challenged TS/A-pc latency time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>None - 0/12 (0%)</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>D5.40</td>
<td></td>
<td>D5.40 Normal rat Ig (100%)</td>
<td>12/12 (100%)</td>
</tr>
<tr>
<td>D5.40 Anti-granulocytes</td>
<td></td>
<td>D5.40 Anti-granulocytes (50%)</td>
<td>6/12(100%)</td>
</tr>
<tr>
<td>D5.40 Anti-CD4 (33%)</td>
<td></td>
<td>D5.40 Anti-CD4 (0%)</td>
<td>4/12(100%)</td>
</tr>
<tr>
<td>D5.40 Anti-CD8 (0%)</td>
<td></td>
<td>D5.40 Anti-CD8 (0%)</td>
<td>0/11(0%)</td>
</tr>
</tbody>
</table>

*As in Table VII.

---

**Table IX.** Adoptive transfer to sublethally irradiated mice of immune memory to TS/A-pc established after the rejection of D5.40 cells

<table>
<thead>
<tr>
<th>T-Spc from Mice Challenged with D5.40</th>
<th>Serum Injection of Normal or Immune Serum</th>
<th>Surviving/Total Mice Challenged with TS/A-pc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nothing</td>
<td>none</td>
<td>0/16 (0%)</td>
</tr>
<tr>
<td>Nothing</td>
<td>normal</td>
<td>0/6 (0%)</td>
</tr>
<tr>
<td>Nothing D5.40 immune (100%)</td>
<td>1/6 (16%)</td>
<td></td>
</tr>
<tr>
<td>D5.40</td>
<td>D5.40 immune 1 × adsorbed</td>
<td>0/6 (0%)</td>
</tr>
<tr>
<td>D5.40</td>
<td>none</td>
<td>2/6 (25%)</td>
</tr>
<tr>
<td>D5.40</td>
<td>normal</td>
<td>1/6 (16%)</td>
</tr>
<tr>
<td>D5.40</td>
<td>D5.40 immune 1 ×</td>
<td>15/16 (94%)</td>
</tr>
<tr>
<td>D5.40</td>
<td>D5.49 immune 1 × adsorbed</td>
<td>2/6 (25%)</td>
</tr>
</tbody>
</table>

*a* Donor mice were challenged in the left flank with nothing or 10⁵ D5.40 cells, and 30 days later 10⁷ T-Spc from tumor-free mice were adoptively transferred i.v. into 5-Gy irradiated recipient mice.

*b* 0.2 ml of normal or immune serum was injected i.v. into recipient irradiated and reconstituted mice 1 and 3 days after TS/A-pc challenge.

---

**FIGURE 8.** IL-4 and IFN-γ production during an 18-h stimulation with anti-CD3 mAb by inguinal lymph node cells from mice primed 10 days earlier with a B6.3600, D5.40, or Mit-C TS/A-pc challenge. Left panel: Cytokine production by lymph node cells from primed mice cultured for 6 days with Mit-C TS/A-pc. Right panel: IFN-γ production by lymph node cells from mice primed with B6.3600 (aZ), D5.40 (AZ), and Mit-C TS/A-pc (μZ), rechallenged with TS/A-pc, and killed 4, 24, and 48 h afterward.
more efficient than those from normal or Mit-C TS/A-pc-immune mice. It is known that IL-4 augments the cytotoxic capacity of effector cells in ADCC (19), and increases the membrane expression of IgE Fc receptor (38). Ab-directed and T lymphocyte-activated eosinophils, macrophages, and neutrophils can thus form an important effector mechanism.

Lastly, the importance of cytokines present during tumor priming on the selection of the memory mechanisms was rendered evident by the experiments in which T cells from B6.3600- and D5.40-primed mice were either restimulated in vitro or in vivo with TS/A-pc. Priming with both B6.3600 and D5.40 cells induces IL-4 production. However, priming with B6.3600 stimulates a marked production of IFN-γ that is absent in mice primed with D5.40 cells. This “cross-regulatory” or inhibitory effect of tumor priming in the presence of local IL-4 on IFN-γ production (25) results in marked imbalance of the cytokines that promote the establishment of a Th2 response. The effect of priming with tumor cells releasing various cytokines on the Th1 and Th2 memory mechanisms is currently under investigation in our laboratory.

The evidence that strong nonspecific and specific reactions follow a tumor cell challenge in the presence of localized cytokines lies behind current proposals for their use as components of new tumor vaccines (6-13). The Th2 features of the immune memory acquired after D5.40 priming in the presence of local IL-4 on IFN-γ production (25) results in marked imbalance of the cytokines that promote the establishment of a Th2 response. The effect of priming with tumor cells releasing various cytokines on the Th1 and Th2 memory mechanisms is currently under investigation in our laboratory.

Acknowledgments
We thank Dr. Dusty Miller for his generous gift of LXSN retroviral vector and Dr. John Iliffe for careful review of the manuscript.

References


