Specific Immunosuppressive Activity of Epiglycanin, a Mucin-like Glycoprotein Secreted by a Murine Mammary Adenocarcinoma (TA3-HA)

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ABSTRACT

Epiglycanin (Epi) is a mucin-like glycoprotein carrying multiple Thomsen Friedenreich (TF) and Tn determinants secreted by a murine mammary adenocarcinoma, TA3-Ha. As an attempt to further characterize immunoregulatory networks in the TA3-Ha animal model, we tested whether Epi causes active suppression of the cell-mediated immune response against TF determinants. In this study, we show that (a) s.c. injection of epiglycanin emulsified in either complete Freund's adjuvant or Ribi adjuvant containing trehalose dimycolate and monophosphoryl lipid A elicits a classical specific delayed-type hyperactivity response to TF hapten either naturally expressed on epiglycanin or as synthetic haptens conjugated to a protein carrier; (b) i.v. injection of as little as 500 ng of Epi induces specific immunosuppression to anti-Epi and anti-TF synthetic antigen delayed-type hyperactivity responses; (c) this immunosuppression can be abrogated by i.v. injection of cyclophosphamide prior to immunizations; (d) Epi-induced specific immunosuppression can be adoptively transferred by nylon wool-nonadherent cells 6 days following i.v. injection of Epi; (e) pretreatment of suppressor cell populations with anti-Thy-1, anti-L3T4, anti-Lyt-1, or anti-I-Jk but not anti-Lyt-2 monoclonal antibodies plus complement prior to adoptive transfer abolished immunosuppression; (f) i.v. injection of immunosuppressive amounts of Epi on Days 2 and 6 after transplantation of TA3-Ha cells increased the lethality of the tumor transplant. These results suggest that Epi-induced specific immunosuppression in the TA3-Ha animal model is mediated by Thy-1+, L3T4+, Lyt-1+, and I-Jk+ suppressor cells. The results are also consistent with the suggestion that this immunosuppression may enhance TA3-Ha tumor growth.

INTRODUCTION

We have recently (1–4) developed an animal model for ASI3 using the murine mammary adenocarcinoma cell line TA3-Ha (5, 6). The TA3-Ha cell line, derived originally from a spontaneous tumor arising in an A/J mouse, produces a glycolyx containing a mucin called epiglycanin (7, 8). Epi has an approximate molecular weight of 500,000 and is composed of 75–80% carbohydrate containing repeating TF (9) and Tn determinants. The TA3-Ha model has many similarities to human adenocarcinomas (4) including the secretion into body fluids of mucins which carry altered carbohydrate determinants including the TF antigen (10, 11) and its immediate precursor, Tn.

Many investigators have suggested that Epi associated with the TA3-Ha tumor is responsible for its extremely high virulence (12). The TA3 mammary carcinoma arose spontaneously in a female A/HeHa mouse (13) and the strain-nonspecific subline, TA3-Ha, was derived from a strain-specific subline, TA3-St (5, 12, 13). The ability of TA3-Ha tumor cells to grow even in foreign, H-2-incompatible mouse strains has been attributed to the presence of a thick glycolcalyx composed primarily of Epi which has been shown to block the binding of anti-H-2 antibodies to the cell surface of TA3-Ha cells (12, 14–16). Although there was no previous evidence for an immunosuppressive role of shed, circulating Epi, others have suggested that serum Epi may form blocking factors (17) in allogeneic mouse. In support of this possibility it was observed that cell-free ascites fluid of TA3-Ha cells growing in mice of foreign strains stimulates the growth of this tumor in normal mice (18).

In our ASI model (4) we have used S-TAGs containing TF hapten as the main immunogenic epitopes conjugated to the carrier protein KLH emulsified in Ribi adjuvant to immunize TA3-Ha tumor-bearing mice. This basic “vaccine” formulation provided approximately 25% long-term survival in groups of mice bearing an otherwise 100% lethal tumor transplant. Less effective formulations which used purified Epi or X-irradiated TA3-Ha tumor cells emulsified in Ribi adjuvant provided no protection (4). However, when administration of the basic “vaccine” formulation was preceded by treatment with low dose Cy up to 90% long-term survival was achieved (4). On the basis of these observations we have postulated (4) that Epi secreted by growing TA3-Ha tumors might induce a class of suppressor cells which render most attempts to achieve successful ASI relatively ineffective. Direct evidence demonstrating a potential immunomodulatory effect of cancer-associated mucins is lacking, although high levels of serum mucins have been shown to correlate with poor prognosis in human cancer patients (19–21). In the present report, we show that i.v. injections of low doses of Epi induce a state of specific immunosuppression for DTH reactions to Epi and its associated TF epitopes. Evidence is also presented that Epi induces a population of cyclophosphamide-sensitive T-suppressor cells. Finally, it was demonstrated that i.v. injections of immunosuppressive quantities of Epi can enhance the lethality of TA3-Ha tumor transplants.

MATERIALS AND METHODS

Mice. Eight- to 10-week-old pathogen-free female CAF1/J mice purchased from The Jackson Laboratory were used throughout this investigation.

Tumor Cell Line. The TA3-Ha tumor cell line was originally provided by Dr. J. F. Codington (Massachusetts General Hospital, Boston, MA). The tumor cells were grown in vivo by weekly passage (i.p.) in CAF1/J mice.

S-TAG and Control Antigens. TF-hapten [β-Gal(1→3)αGalNAc] conjugated to KLH or HSA were obtained from Biomira, Inc., Edmonton, Alberta, Canada. KLH was purchased from Calbiochem (La Jolla, CA). BSA and HSA was purchased from Sigma Chemical Co. (St. Louis, MO).
Purification of Epi. Ascites from BALB/c mice bearing TA3-Ha i.p. tumors was collected and cells were removed by centrifugation. Epi from ascites was extracted on a peanut agglutinin lectin column as described previously (1). The Epi preparation was characterized as a single peak by fast protein liquid chromatography and the Epi activity was detected and standardized with 125I-labeled monoclonal antibody, 49H.8 (22), and 125I-labeled peanut agglutinin in a direct as well as a sandwich radioimmunoassay (11). The lot of Epi used in these studies had a carbohydrate:protein ratio of approximately 79:21 as determined by the procedure of Lowry et al. (23), for the protein estimate and the phenolsulfuric acid method of Dubois et al. (24) for the carbohydrate content. The amino acid composition of our preparation of Epi determined by the method of Moore (25) was found to be very similar to the published amino acid composition (12) of Epi containing approximately 50% serine and threonine residues and approximately 9% proline, characteristic of O-linked mucin-like molecules. The amount of Epi used in this study was estimated using a quantitative sandwich radioimmunoassay (11).

Induction of DTH Responses to Epi and S-TAGs. Mice were given injections of 450 ng Epi or 50 μg S-TAG emulsified in 50% CFA or 0.2 ml Ribi adjuvant (containing trehalose dimycolate and monophosphoryl lipid A) purchased from Ribi Immunocoach Research, Inc., Hamilton, MT. The emulsion was distributed equally among two s.c. sites in the upper belly and one site at the base of the tail. Seven days after immunization DTH was evaluated [as described previously (1)] following injection into the footpad of 450 ng Epi or 50 μg S-TAG or control antigens. Footpad thickness was measured with a vernier caliper. The results, expressed as net footpad swelling, were calculated as the difference in footpad thickness of test antigen and sterile saline-injected pads at various time intervals minus the difference in footpad thickness of test antigen and sterile saline-injected pads before footpad challenge.

Induction of Immunosuppression. Mice were given i.v. injections of various concentrations of Epi dissolved in sterile saline. Six to 7 days later, the mice were immunized with Epi + CFA or Epi + Ribi adjuvant, or S-TAGs emulsified in Ribi adjuvant in the manner described above and 7 days later were footpad tested for DTH responsiveness to Epi or S-TAGs. To test the immunosuppression induced by control antigens such as BSA or KLH, separate groups of mice were given i.v. injections of either 200 μg BSA or KLH in sterile saline. These animals were immunized and footpad tested with Epi, BSA, or KLH in a similar manner.

Adoptive Transfer of Suppressor Cells. Spleens were collected from mice 6 to 7 days after i.v. injection of Epi. Single cell suspensions were prepared by pressing tissues through a 100 mesh stainless steel screen in phosphate buffered saline. Spleen cells were treated with ammonium chloride-Tris-buffered solution to lyse the erythrocytes. The cells were washed three times, counted, and resuspended in phosphate buffered saline at a concentration of 6.4 × 10^7 cells/ml.

To determine the effects of suppressor cells on the induction of DTH responses, adoptive transfers were done on the day of immunization of the recipients. These animals were tested for DTH responsiveness 7 days later.

Antisera Treatment of Cells. Anti-Thy-1.2, anti-L3T4, anti-Lyt-2, and anti-I-Jk monoclonal antibodies (kindly provided by Dr. D. Green, Department of Immunology, University of Alberta) and low toxicity rabbit complement (diluted 1:10; Cedarlane Laboratory, Hornby, Ontario, Canada), were used for cytotoxic depletion of cells from splenic populations. Anti-Lyt-1.2 monoclonal antibody was purchased from Cedarlane laboratory and was used at a final dilution of 1:10. Enrichment for T-lymphocytes on nylon wool columns was performed according to previously described procedures (26).

CY Treatment. In an attempt to reverse Epi-induced immunosuppression, Epi- or saline-treated mice were treated i.v. with 100 mg/kg CY (Sigma) prior to immunization with appropriate antigens.

Statistical Analysis. Student's t test was used to test differences in DTH reactivity among groups and modified Wilcoxon was used to evaluate survival differences.

RESULTS

Induction and Specificity of DTH Responses Elicited by Epiglycanin and S-TAGs. We performed studies to establish the kinetics of a direct DTH reaction to epiglycanin following s.c. immunizations with Epi emulsified in appropriate adjuvants (Fig. 1). Maximum DTH reactions occurred between 24 and 30 h in Epi + CFA- or Epi + Ribi adjuvant-immunized mice. No obvious early peak of DTH reactivity was observed between 2 and 6 h after antigen challenge as was observed in other antigenic systems (27). The DTH reactivities were still high at 48 h and by 72 h they started to subside. By 96 h, the swellings decreased to an undetectable level. The kinetics of this DTH is different from that of an antibody-mediated Arthus reaction in which a single peak swelling is observed 4–10 h after antigen challenge and similar to our previous data using an adoptive transfer system for DTH (1) to Epi and S-TAGs. Mice immunized with nonspecific control antigens in CFA or Ribi adjuvant always gave negligible footpad swelling (data not shown). In separate experiments (data not shown) we showed that DTH reactions to Epi are transferable with immune spleen T-cells which is in agreement with our previous studies (1).

Table 1 shows that Epi-induced DTH reactions can be specifically directed to TF (α) epitopes, again confirming our previous specificity studies using an adoptive transfer system (1). Mice immunized with Epi s.c. were able to mount a strong DTH response to Epi or the TF-α-HSA glycoconjugate, but not to the carrier protein HSA. Using the tumor-associated glycoconjugate (TF-α-KLH) as an immunizing antigen, we can also generate a specific DTH reaction to the TF-α determinant (Table 2).

Induction of Immunosuppression. The next series of experiments were designed to investigate the effect of i.v. administration of Epi on DTH responses. Fig. 2 shows that mice receiving i.v. preinjections of Epi had a dose-dependent reduction in their
DTH reactivities to Epi. It was found that as little as 500 units (=500 ng) still produced significant suppression. The next experiment examines the specificity of induction of immunosuppression by unrelated antigens. Control mice which were given i.v. injections of either sterile saline, BSA, or KLH and later immunized with Epi plus Ribi adjuvant s.c. all elicited a strong DTH reaction at 24 h (Fig. 3). When mice were given saline, compared with group 1; d, compared with Group 4; e, footpad tested with 50 μg BSA, compared with Group 6; f, footpad tested with 50 μg KLH, compared with Group 9. NS, nonsignificant.

Adoptive Transfer of Immunosuppression by Spleen Cells. To investigate the possibility that Epi can induce active suppression, we collected spleen cells from donor mice given i.v. injections of 1.8 μg Epi 6–7 days earlier. The cells were transferred to normal syngeneic mice at the same time the recipients were immunized. Seven days later, the recipient animals were footpad challenged, and footpad thicknesses were measured 24 h after challenge. Cells capable of suppressing the DTH response following adoptive transfer were detected in the spleens of Epi-treated mice while spleen cells from control mice had no significant effect on the DTH response of the recipients (Table 3).

Specificity Studies of Cells Mediating Active Suppression. To assess the specificity of active suppression, spleen cells from Epi-treated mice were adoptively transferred to naive mice at the time of immunization with TFα-KLH+Ribi. Seven days after immunization, each group of mice was footpad challenged with the homologous antigen, the carrier protein as well as with TF antigen conjugated on HSA, a different protein carrier. Fig. 4 shows that nylon wool-enriched T-cells from mice treated with Epi inhibited the DTH response to TFα-HSA by 47% (P < 0.05) whereas the same cell population exerted no effect on DTH response to the protein carrier KLH.

Characterization of the Cells Mediating Active Suppression. Data from Fig. 4 show that the Epi-induced spleen cells mediating suppression were present in the nylon wool-nonadherent fraction which presumably contain T-cells. To further characterize these cells, experiments were done to establish their surface phenotype by a cytotoxic antibody depletion technique. Recipients of untreated or antibody-treated cells were immunized on the day of cell transfer and footpad challenged 7 days later. Treatment of cells before adoptive transfer with

Table 2 Direct DTH responsiveness to the TFα-hapten following immunization with TFα-KLH

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Footpad antigen</th>
<th>Net footpad swelling (mm ± SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 μg TFα-KLH emulsified in 0.2 ml Ribi adjuvant</td>
<td>TFα-KLH</td>
<td>0.38 ± 0.10 0.27 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>KLH</td>
<td>0.71 ± 0.20 0.42 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>HSA</td>
<td>0.02 ± 0.02 0.00 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Epi</td>
<td>0.35 ± 0.05 0.25 ± 0.05</td>
</tr>
</tbody>
</table>

* Average of three mice. All mice were footpad tested for DTH 7 days after immunization.

Table 3 Adoptive transfer of immunosuppression of DTH responsiveness by spleen cells

<table>
<thead>
<tr>
<th>Treatment of mice</th>
<th>Tenth treatment (Day 0)</th>
<th>DTH response to immunogen (mm ± SD)*</th>
<th>% Suppression</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Saline</td>
<td>Epi + CFA</td>
<td></td>
<td></td>
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<tr>
<td>Group 2</td>
<td>0.9 μg Epi</td>
<td>Epi + CFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>1.8 μg Epi</td>
<td>Epi + CFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 4</td>
<td>Saline</td>
<td>Epi + Ribi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 5</td>
<td>1.8 μg Epi</td>
<td>Epi + Ribi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 6</td>
<td>Saline</td>
<td>BSA + Ribi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 7</td>
<td>200 μg BSA</td>
<td>Epi + Ribi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 8</td>
<td>Saline</td>
<td>KLH + Ribi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 9</td>
<td>200 μg KLH</td>
<td>Epi + Ribi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 10</td>
<td>200 μg Epi</td>
<td>Epi + Ribi</td>
<td></td>
<td></td>
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</tbody>
</table>

* Mice were immunized s.c. with 450 ng Epi emulsified in CFA. ** Average of five mice. All mice were footpad tested for DTH responsiveness to Epi (450 ng/footpad) 7 days after immunization.
complement alone or anti-Lyt-2 plus complement had no effect on the ability of Epi-induced spleen cells to inhibit the DTH response (Fig. 5, Groups 3 and 7), whereas treatment with anti-Thy-1, anti-L3T4, anti-Lyt-1.2, or anti-I-JK antibodies plus complement abolished the suppressive activity of the cells (Groups 4, 5, 6, and 8 compared with Groups 2 and 3). These results indicate that the cells mediating active suppression in this system are Thy-1+, L3T4+, Lyt-1.2+, and I-J+ T-lymphocytes.

Abrogation of Immunosuppression following CY Treatment. CY has been shown to inhibit the development of active suppression mediated by T-cells in experimental systems, thereby allowing the host to generate an augmented level of immunity to a variety of antigens. Thus we investigated whether CY pretreatment would restore the DTH response in Epi-treated mice. CY was injected i.v. at a dose of 100 mg/kg 1 day prior to immunization with appropriate antigens. The animals were then footpad challenged in the usual manner 7 days after immunization. Table 4 shows that the Epi-induced DTH responses in mice given i.v. injections of Epi were significantly depressed (82.4 and 81.9% suppression, P < 0.005), whereas Epi-treated mice which were subsequently given injections of CY 24 h prior to immunization recovered their DTH reactivities (19 and 47.6% enhancement).

Effect of i.v. Epi Injections on Survival of TA3-Ha Tumor-bearing Mice. We used our new radioimmunoassay (11) to monitor the presence of Epi in the circulation of TA3-Ha-bearing mice. CAF1 mice were given i.p. injections of approx.

- 40, 23, 20, 23, and 24%, respectively. All mice were footpad tested with 450 ng Epi, 24-h. readings.
- treatment with sterile saline or 1.8 ng Epi in a volume of 0.4 ml; 3 mice/group, b, 6 days after i.v. treatments, spleen cells pooled from each group of animals and 0.5-1.0 × 10^6 cells were treated with monoclonal antiserum plus complement (C') or complement alone prior to cell transfer. Percentage of cytotoxicity of cells after antiserum treatment with Thy-1, L3T4, Lyt-1.2, Lyt-2, and I-Jk were 40, 23, 20, 23, and 24%, respectively. c, all mice footpad tested with 450 ng Epi; d, P compared to Group 1. NS, nonsignificant.

**Fig. 5.** Characterization of Epi-induced spleen cells mediating active suppression. a, mice given injections of either sterile saline or 1.8 μg Epi in a volume of 0.4 ml; 3 mice/group, b, 6 days after i.v. treatments, spleen cells pooled from each group of animals and 0.5-1.0 × 10^6 cells were treated with monoclonal antiserum plus complement (C') or complement alone prior to cell transfer. Percentage of cytotoxicity of cells after antiserum treatment with Thy-1, L3T4, Lyt-1.2, Lyt-2, and I-Jk were 40, 23, 20, 23, and 24%, respectively. c, all mice footpad tested with 450 ng Epi; d, P compared to Group 1. NS, nonsignificant.

**Table 4** Effect of cyclophosphamide treatment on immunosuppression

<table>
<thead>
<tr>
<th>Treatment of mice</th>
<th>DTH responses to Epi (Day 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>24 h reading of mean increase</td>
</tr>
<tr>
<td></td>
<td>of footpad thickness (mm ± SD)</td>
</tr>
<tr>
<td></td>
<td>% Suppression (P) Value</td>
</tr>
<tr>
<td>1 Saline</td>
<td>-</td>
</tr>
<tr>
<td>2 Epi</td>
<td>+</td>
</tr>
<tr>
<td>3 Epi C'</td>
<td>+</td>
</tr>
<tr>
<td>4 Epi Thy-1×C'</td>
<td>+</td>
</tr>
<tr>
<td>5 Epi L3T4×C'</td>
<td>+</td>
</tr>
<tr>
<td>6 Epi Lyt-1.2×C'</td>
<td>+</td>
</tr>
<tr>
<td>7 Epi Lyt-2×C'</td>
<td>+</td>
</tr>
<tr>
<td>8 Epi I-J×C'</td>
<td>+</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Many studies have shown that tumor-associated mucins can be detected in the sera of most adenocarcinoma patients (19, 20, 28-32) and that high levels of cancer-associated mucins appear to correlate with poor prognosis in some human cancer patients (20, 21). Steck and Nicolson (33) observed that the concentration of a mucin-like cell surface glycoprotein (M, 580,000) was correlated with the metastatic potential in a series of clones of rat mammary adenocarcinoma cells. The expression of a sialomucin on rat mammary tumor cells has been correlated with natural killer cell susceptibility (34). In breast cancer patients increased expression of TF antigen has been correlated with tumor progression and metastatic spread (35). Many additional studies suggest that TF antigen expression is associated with poor prognosis or malignant change (36-43). These and other findings suggested to us that circulating mucins in general and TF-bearing antigens in particular might inhibit the host's anti-tumor immune response. This prompted us to investigate whether mucins might inhibit cell-mediated immune responses in our TA3-Ha model. The TA3-Ha tumor produces a glyco-calyx containing a mucin called epiglycanin (7, 8), a M, 500,000 glycoprotein composed of 75-80% carbohydrate. Epi contains multiple TF and Tn determinants which are also expressed on over 90% of human adenocarcinomas (9) as well as on mucin-like molecules in body fluids of adenocarcinoma patients (10, 11). Epiglycanin is also secreted into body fluids (17). In order to address the question of how the presence of mucins in body fluids might affect the immune function of the host, we injected i.v. various concentrations of epiglycanin purified from TA3-Ha tumor ascites fluid and assessed the effect on subsequent DTH responses elicited against the homologous epiglycanin.
antigen or TF hapten conjugated to a protein carrier. We have found that i.v. injected Epi induces specific immunosuppression as well as cells capable of specifically inhibiting DTH reactions in response to both Epi and the TF-α epitope. The cells mediating active suppression were detectable in the spleen 6 days after Epi injection. Immunosuppressive activity in this system was shown to be hapten specific since adoptive transfer of the spleen cells mediating suppression inhibited the induction of the DTH response to the TF-α hapten but had no effect on the response to the protein carrier KLH. However, when control antigens such as BSA or KLH at a tolerogenic dose were administered i.v. into mice that were immunized s.c. with Epi + Ribi 6 days later, no suppression of the Epi-induced DTH responses was observed, indicating that the induction of immunosuppression of the DTH response observed in this study was mucin induced and antigen specific.

In order to determine the nature of the cells mediating active suppression we passed the splenic cell pools over nylon wool columns to remove the majority of B-cells and macrophages and to enrich for T-cells prior to cell transfer. Using this procedure, we ascertained that the nylon wool-nonadherent cell populations from Epi-treated mice contained the suppressor cells. These results suggested that the cells responsible for the suppression were T-lymphocytes. When the suppressive splenic cell populations were treated with anti-Thy-1 antibody plus complement prior to adoptive cell transfer, the suppressive activity was ablated, confirming that T-lymphocytes were required for the activity. Abrogation of the suppressive activity by treatment of the spleen cells with anti-L3T4, anti-Lyt-1.2, or anti-I-J* antibody plus complement but not with complement alone or those treated with anti-Lyt-2 and complement was observed, indicating that the cells mediating the suppression were L3T4+, Lyt-12*, and I-J* T-cells.

Antigen-specific T-cells which suppress humoral and cell-mediated immune responses (44, 45) in many other systems have been described. Such suppressor cell systems include those that regulate the DTH responses to azobenzene arsencate (46) and those that regulate the DTH and/or the antibody responses to 4 hydroxynitrophenylacetyl (47, 48) and sheep erythrocytes (44). Suppressor T-cells can also be demonstrated to inhibit antitumor immunity (49, 50). Recently, Takahashi et al. (51) demonstrated that in B16 melanoma system, suppressor T-cells generated by the soluble form of a tumor-associated antigen were able to block the induction as well as the effector phase of cytotoxic T-lymphocytes. Analysis of known antigen-specific suppressor cells revealed that at least two populations of suppressor T-lymphocytes are involved in immune regulation (51). Induction-phase suppressor T-cells (TS1), which can be generated by antigen alone has the phenotype of L3T4+, Lyt-12*, and I-J*, whereas the effector-phase T-suppressors (TS2), which depend for their generation on both antigen and TS1, were L3T4+, Lyt-21*, and I-J*. The suppressor T-lymphocytes identified in this study, shown to have a suppressive effect on the induction phase of specific DTH response to Epi and TF-α epitopes, were Thy1+, L3T4+, Lyt-12*, and I-J*K+ T cells and thus resembled the TS1 suppressor described in other antigen-specific suppressor cell systems (44, 45).

Codiumon et al. (10) demonstrated that many patients with high concentrations of epiglycanin-like antigen suffer from advanced metastatic cancer. Furthermore, it has been reported that the metastatic potential in various TA3-Ha ascites variants was proportional to the epiglycanin concentration (16). Others have suggested that shed Epi might be immunosuppressive (17, 18). Our data show that Epi molecules are highly immunosuppressive for specific DTH reactions following their introduction into blood circulations. This suggested to us that tumor-associated mucins which are shed into the blood circulation might accelerate the disease. In support of this hypothesis we have found that i.v. injections of immunosuppressive amounts of Epi increased the lethality of the TA3-Ha tumor transplant.

Early studies in mice have shown that doses of antigen above or below the usual immunizing range tend to induce high and/or low zone tolerance, respectively (52–54). Injecting antigen i.v. has also been shown to be a way of providing a tolerogenic signal (55). A dose of 10–40 µg BSA and those of 5 mg or above were effective in inducing a low and high zone tolerance in mice, respectively (52). In the present studies, we have demonstrated that i.v. injection of a very low dose of Epi (500 ng) is immunosuppressive for DTH reactions to Epi and its associated TF epitopes. Because of previous work demonstrating the immunosuppressive properties of heterologous proteins injected i.v. (52–55) it might be argued that there is nothing unique about the immunosuppressive properties of Epi. However, we believe that the present work should be viewed in the context of the important biological questions which are being asked. Do mucin molecules which are normally sequestered in secretory granules or on mucosal surfaces but which are secreted by most adenocarcinomas in altered form into the blood circulation have an effect on the immune response to the important cancer-associated carbohydrate epitopes that they carry? This question assumes even greater importance if one considers that high mucin levels and/or high TF antigen expression often correlates with poor prognosis or rapid tumor progression (10, 20, 21, 33–43) despite the fact that TF on various natural or synthetic glycoconjugates can be highly immunogenic (1–4, 9, 56–61) and that cancer-associated carbohydrate antigens can apparently serve as effective targets for the immune destruction of tumor cells which carry those antigens (1–4, 62–65). A logical conclusion is that the immune response to this class of cancer-associated antigens is ineffective and/or suppressed. The present communication provides a rationale for the latter conclusion but does not rule out the former.

In another study of active specific immunotherapy of mice bearing TA3-Ha tumor transplants (4) we failed to provide any protection of animals which had received subsequent active immunizations with Epi or irradiated whole TA3-Ha tumor cells emulsified in Ribi adjuvant. We suggested that this failure may have been due to an active immunosuppression induced by circulating epiglycanin shed from the growing tumor and the present report supports that hypothesis. The suggested mechanism predicts that pretreatment with CY, an agent which at an appropriate dosage can preferentially inhibit the development of suppressor T-cells (66–68), should provide relief of immunosuppression in the tumor-bearing animals. In fact in the same study we were able to show that tumor-bearing animals which were treated with low dose CY followed by active immunizations with TF-α-KLH were able to achieve up to 90% long-term survival. In the present study, pretreatment of epiglycanin-treated animals with CY also led to a successful recovery of their DTH reactivities to the immunizing agent. This adds further support to the potential therapeutic effect of CY in the active specific immunotherapy model. Our previous work (1) demonstrated that effector cell populations with a T-helper phenotype which showed a strong DTH reactivity to TF epitopes also gave specific antitumor effects when tested with TA3-Ha tumor cells in a Winn-type assay. At present we are studying
these effector T-cell populations at the clonal level and plan to investigate the effect of suppressor cell populations on their expression.

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REFERENCES


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