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Immunology

Inhibitors of glycoprotein processing alter T-cell proliferative responses to antigen and to interleukin 2

(swainsonine/castanospermine/T-cell receptor)

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ABSTRACT **Most of the cell-surface molecules involved in T-cell immune responses are N-linked glycoproteins. We have investigated the effects of inhibitors of glycoprotein processing on specific T-cell functions, with the dual aims of examining the functional role of carbohydrate and of testing the usefulness of such compounds as immunomodulators. Treatment of a cloned murine helper T-cell line with these inhibitors differentially affects the proliferative response of the cell, depending upon the nature of the stimulus. Treatment with the plant alkaloid swainsonine, which inhibits the processing mannosidase II and causes the accumulation of glycoproteins bearing hybrid-type oligosaccharide structures, enhances the proliferative response of the T-cell clone to antigen and to the mitogen concanavalin A. Treatment with another plant alkaloid, castanospermine, which inhibits glucosidase I and causes the accumulation of glucose-containing high-mannose structures, has the opposite effect and inhibits the proliferative response of the T cell to antigen. Cell-surface oligosaccharide alteration does not affect antigen recognition, as judged by the lack of effect of either drug on interleukin 2 production following antigen stimulation. Cells treated with either alkaloid proliferate poorly to exogenous interleukin 2 and may have defective interleukin 2 receptor function. Swainsonine-treated cells apparently have compensatory alterations that can overcome the reduced responsiveness to interleukin 2. Antibody-binding studies indicate that normal quantities of many cell-surface molecules, including the T-cell receptor for antigen, are expressed by the treated cells.**

Immune system responses mediated by T cells are largely controlled by the involvement of surface glycoproteins in antigen recognition and subsequent triggering of cell functions. Some functions are mediated by or dependent on secreted factors such as lymphokines, which are also glycoproteins. The role of the carbohydrate portion of these cell-surface and secreted molecules in the function of immune cells is not well understood but is of considerable interest in understanding how these molecules work at the molecular level. In the past, the question of whether carbohydrate contributed to a glycoprotein function was approached by treatment of the cell with tunicamycin, an antibiotic that blocks N-linked glycosylation by preventing formation of the first lipid intermediate, GlcNAc-diphosphodolichol (1). For example, Kornbluth (2) demonstrated a loss of cytolytic T-cell function following treatment of T cells with tunicamycin. In many cases the use of tunicamycin leads to confusing results, since a total lack of glycosylation can lead to accumulation of partially synthesized molecules in the endoplasmic reticulum and to loss of expression of the molecule itself.

We have taken advantage of the existence of a variety of drugs that inhibit enzymes in the glycoprotein-processing pathway causing cells to produce altered oligosaccharide structures in their N-linked glycoproteins, yet without greatly altered levels of glycoprotein expression. The oligosaccharide portion of the N-linked glycoprotein is initially assembled on a lipid carrier as a $Glc_3Man_9(GlcNAc)_2$-diphosphodolichol. The oligosaccharide, $Glc_3Man_9(GlcNAc)_2$, is transferred to certain asparagine residues on the protein, and then the oligosaccharide undergoes a number of enzymatic processing reactions (3). Thus, some sugars are removed and others are added to sequentially produce high-mannose, hybrid, or complex types of oligosaccharides (4). We have previously demonstrated that castanospermine, a plant alkaloid, is an inhibitor of glucosidase I and prevents the removal of the glucose residues from the N-linked core oligosaccharide (5, 6). This removal is the first step in posttranslational processing and is necessary for this oligosaccharide to mature and be converted to the sialic acid-containing complex chains. Swainsonine, another plant alkaloid, is an inhibitor of some α-mannosidases and prevents the removal of certain mannose residues from the oligosaccharide chains after they have already undergone some modifications (7–9). The predominant site of inhibition is the processing mannosidase II. In the presence of this inhibitor, cells produce hybrid types of oligosaccharide structures that have unusual and immature carbohydrate chains. We find that treatment of helper T cells with these inhibitors can cause enhancement or diminution of T-cell responses, depending on the nature of the stimulus.

MATERIALS AND METHODS

Chemicals. Castanospermine was isolated from the seeds of *Castanospermum australe* as described (10). Briefly, the alkaloid was extracted from pulverized seeds with 70% methanol and purified by ion-exchange chromatography and crystallization from ethanol. Swainsonine was isolated from the leaves and stems of *Astragalus lentiginosus* (locoweed) by methanol extraction as described (11). Purification was by ion-exchange chromatography, thin-layer chromatography, and sublimation.

Cell Lines. Helper T-cell clone 46 was isolated by limiting dilution from an A.TH anti-A.TL mixed lymphocyte culture (MLC). The procedure of Glasebrook and Fitch (12) was used for maintenance of the cell line on CBA/J (Ia^k) spleen cells. Routine transfer cultures were supplemented with supernatant liquid from secondary MLC or from concanavalin A (Con A)-stimulated Lewis rat spleen cells (LCA), prepared as described (12). Culture medium was Dulbecco's modified Eagle's medium supplemented with 116 mg of arginine per liter, 36 mg of asparagine per liter, 216 mg of glutamine per liter, 110 mg of pyruvate per liter, 6 mg of folic acid per liter, 10% fetal calf serum, 10 mM Mops, 0.05 mM 2-mercapto-

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Abbreviations: IL-2, interleukin 2; IL-4, interleukin 4; LCA, Lewis rat spleen cells stimulated with Con A; MLC, mixed lymphocyte culture.

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ethanol, 10^5 units of penicillin per liter, and 100 mg of streptomycin per liter. Helper T-cell clone L2 was derived from a C57BL/6J anti-DBA/2J MLC as described (12) and recognizes the murine antigen $Mls^{a,d}$ in the context of nonpolymorphic Ia determinants (13). Both T cells secrete interleukin 2 (IL-2) in response to antigenic stimulation.

Monoclonal antibodies were used as culture supernatants from growth of hybridomas producing the following antibodies: ST44 (K.A.W., unpublished results), rat anti-Thy-1; 7D4 (14), rat anti-IL-2 receptor; GK1.5 (15), rat anti-L3T4; 441.8 (16), rat anti-LFA-1; and KJ16 (17), mouse anti-T-cell receptor allotype.

Assays. Proliferation of cloned T cells was assayed by culturing a fixed number of cloned T cells (4×10^4) and fresh, irradiated (1500 rad; 1 rad = 0.01 gray) stimulator spleen cells (10^6) in microtiter wells (Costar 3596, Costar, Cambridge, MA) in the absence of exogenous IL-2. After 66 hr at 37°C and 5% CO_2, the cells were pulsed with 1 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine per well (2 Ci/mmol; ICN, Costa Mesa, CA). Six hours later the cells were harvested onto a glass fiber filter (Mini Mash II, M. A. Bioproducts, Walkersville, MD). The dried filters were assayed for radioactivity in the presence of scintillant in a Beckman model 6800 liquid scintillation counter. In some cases, Con A or an IL-2-containing supernatant was added or substituted for stimulator cells.

To measure IL-2 production, 3×10^5 T cells were added to 2-ml cultures containing 6×10^6 irradiated spleen cells. Supernatants were harvested at specified times and assayed for IL-2 activity. IL-2 was assayed by using the IL-2-dependent cell line CTLL2 (18). Supernatants of antigen-stimulated cultures were harvested and added in serial dilution to microtiter wells containing 4×10^3 CTLL2 cells. Incorporation of a 6-hr pulse of [^{3}H]thymidine was measured after 24 hr of culture.

For inhibitor pretreatment, 2-ml cultures (Linbro 76-033-05) of cloned T cells were pooled on day 6 or 7 of the weekly transfer cycle. The pooled cells were redistributed into 2-ml wells to ensure the same initial T-cell number per well. Dilutions of swainsonine, castanospermine, or medium were added and the cells were allowed to incubate, usually for 24 or 48 hr. The treated cells were then harvested, diluted to the appropriate final concentration, and assayed.

Binding Analyses. Immunofluorescent staining was performed as described (19). Fluorescein-conjugated MAR18.5, a mouse anti-rat κ monoclonal antibody, was used as the second-step reagent. Stained cells were analyzed on a Becton Dickinson dual-laser FACS IV fluorescence-activated cell sorter with logarithmic amplifier.

Glycopeptide Analysis. Clone 46 cells were precultured with inhibitor for 24 hr in the presence of LCA supernatant. The cells were washed into glucose-free culture medium (Select-Amine, GIBCO) containing the same concentration of inhibitor. [2-^{3}H]Mannose (0.25 mCi per 4×10^6 cells; 10–20 Ci/mmol; American Radiolabeled Chemicals, Saint Louis) was added and allowed to incorporate for 5 hr at 37°C. The cells were then harvested, washed with saline, and digested with Pronase as described (6). Glycopeptides were isolated by passage over a calibrated Bio-Gel (Bio-Rad) P-4 column. The glycopeptide fraction was pooled and applied to a column of Con A-Sepharose. The column was washed with buffer and bound glycopeptides were eluted first with 10 mM α-methyl glucoside and then with 250 mM α-methyl mannoside. The radioactivity in each fraction was determined and total cpm in each group were calculated. The wash contains those glycopeptides that do not bind Con A—i.e., tri- and tetraantennary complex carbohydrates. Biantennary and hybrid-type oligosaccharides are eluted by α-methyl glucoside, whereas high-mannose-type oligosaccharides and some residual hybrid types are eluted by α-methyl mannoside (20).

RESULTS

Cloned T Cells Pretreated with Inhibitors of Glycoprotein Processing Show Altered Proliferation to Antigen. A large number of the T-cell proteins involved in the response to antigen are cell-surface glycoproteins. To investigate whether alterations in glycoprotein processing affected proliferative responses, we pretreated helper T-cell clone 46 with various amounts of the processing inhibitors swainsonine and castanospermine for 24 hr. The treated cells were stimulated with appropriate irradiated stimulator spleen cells in the absence of exogenous IL-2. Proliferation of the clones was determined after 72 hr by incorporation of a 6-hr pulse of [^{3}H]thymidine. Pretreatment with swainsonine enhanced the T-cell proliferative response by ≈3-fold (Fig. 1). The effect was dose dependent, with half-maximal stimulation occurring at 0.005 μg of swainsonine per ml in the pretreatment step. Treatment with swainsonine inhibits the processing mannosidase II and causes an accumulation of hybrid-type oligosaccharides at the expense of complex chains. On the other hand, when the T cells were pretreated with castanospermine, an inhibitor that acts at the glucosidase I stage, the opposite effect was observed. Proliferation was inhibited in a dose-dependent manner (Fig. 1), with total inhibition of proliferation occurring at 200 μg of castanospermine per ml. Cells treated with this alkaloid accumulate glucose-containing high-mannose-type oligosaccharides at the expense of hybrid and complex chains. A similar pattern of stimulation with swainsonine treatment and inhibition with castanospermine treatment was observed with a second T-cell clone, L2 (data not shown). When the pretreatment period was extended from 24 to 48 hr, the same pattern of stimulation and inhibition was observed (data not shown), with the same dose–response curves. Preincubation with alkaloid was required to observe the effect with swainsonine. Thus, when the inhibitors were added to the T-cell-stimulator cell cultures at the initiation of culture (i.e., with no T-cell pretreatment), no effect of swainsonine on T-cell proliferation was observed (Fig. 2). However, a substantial, but reduced, inhibition by castanospermine was observed in the absence of pretreatment (Fig. 2). When the clones were incubated with these same concentrations of swainsonine or castanospermine in the absence of stimulator spleen cells, no proliferation was observed (data not shown).

Alteration of Glycoprotein Structure Occurs Over a Similar Dose Range as the Biological Effect. Although both inhibitors have been shown in other systems to affect glycoprotein

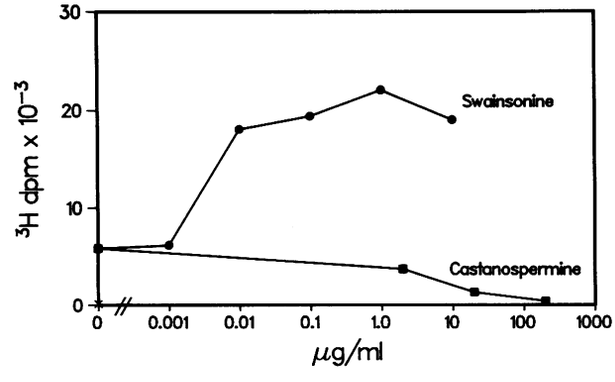

FIG. 1. Dose–response curve for swainsonine and castanospermine. Helper T-cell clone 46 cells were incubated with the indicated concentrations of inhibitors for 24 hr. Cells (4×10^4) were then plated onto 1×10^6 fresh irradiated CBA/J spleen cells in microtiter wells in quadruplicate cultures, which diluted the inhibitor concentration by a factor of 2. After 66 hr, the wells were pulsed with 1 μCi of [^{3}H]thymidine for 6 hr. Incorporation was measured by harvesting the cells onto glass fiber filters and by scintillation spectrometry. ●, Swainsonine; ■, castanospermine; ×, spleen cells alone (200 dpm).

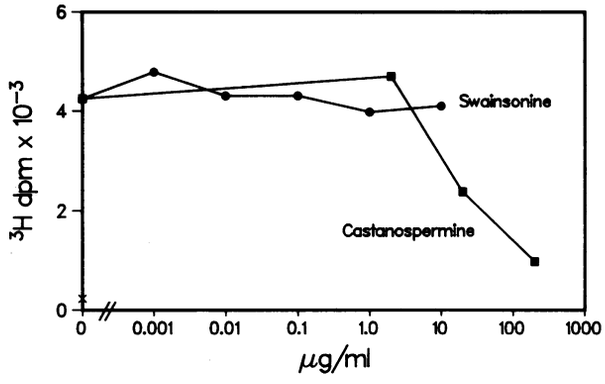

FIG. 2. Dose–response curve for swainsonine and castanospermine with no pretreatment. Untreated clone 46 cells were harvested and plated onto irradiated CBA/J spleen cells as in Fig. 1. Swainsonine and castanospermine were added to the microwells to give the indicated final concentration. [^{3}H]Thymidine incorporation was measured as for Fig. 1. ●, Swainsonine; ■, castanospermine; ×, spleen cells alone (250 dpm).

processing, it was necessary to demonstrate that the effects on cellular proliferation occurred over the same dose range as effects on cellular carbohydrate. Since the exact sites of the biological effects were not known, the alteration of bulk cellular oligosaccharides was determined. Cells treated with swainsonine or castanospermine were biosynthetically labeled with [^{3}H]mannose. Total oligosaccharides were prepared and isolated, and their nature was determined through analysis of binding to and elution from Con A-Sepharose. This procedure fractionates tri- and tetraantennary complex-type oligosaccharides into the wash fraction, biantennary and hybrid-type oligosaccharides into the first eluate, and high-mannose and some hybrid-type oligosaccharides into the second eluate (20). Swainsonine treatment caused a decrease in tri- and tetraantennary oligosaccharides and a corresponding increase in Con A-binding oligosaccharides even at a concentration as low as 10 ng/ml (Table 1). The percentage of Con A-binding oligosaccharides increased with increasing concentrations of swainsonine. With castanospermine treatment, the percentage of oligosaccharides that bind tightly to Con A was measurably changed at 2 μg of the inhibitor per ml. Con A-binding glycopeptides increased with increasing amounts of the inhibitor. Approximately 100-fold more castanospermine than swainsonine was required to produce similar levels of alteration in carbohydrate structure. This may reflect difficulty in cellular uptake of the alkaloid, since castanospermine binds very tightly to isolated glucosidases (5). In both cases, however, the amount of inhibitor needed to observe the predicted carbohydrate modification is in the same concentration range as that required to observe the effect on proliferation.

Table 1. Fractionation of glycopeptides on Con A-Sepharose

Cell treatment	% of total [^{3}H]mannose		
	Wash	α-MeGlc	α-MeMan
No inhibitor	52	12	36
Swainsonine			
10 ng/ml	36	18	46
100 ng/ml	28	15	57
1 μg/ml	21	24	55
Castanospermine			
2 μg/ml	31	23	46
20 μg/ml	17	24	59
200 μg/ml	13	20	67

Pronase digests from [^{3}H]mannose-labeled clone 46 cells were fractionated on Bio-Gel P-4. Each glycopeptide pool was applied to Con A-Sepharose. Following a buffer wash, the bound glycopeptides were eluted first with 10 mM α-methyl glucoside (α-MeGlc) and then with 250 mM α-methyl mannoside (α-MeMan). [^{3}H]Mannose in an aliquot of each fraction was determined and cpm for each group of fractions was calculated. Results are expressed for each group as the % of total cpm in aliquots of the recovered fractions, where 100% ranged from 2378 to 4671 cpm.

The Proliferative Response to Con A Is Increased by Swainsonine and Castanospermine. As expected, clone 46 cells showed increased amounts of Con A-binding glycopeptides when pretreated with either swainsonine or castanospermine (Table 1). Since T cells will respond to Con A as a mitogen, we tested the proliferative response of the treated clone 46 cells in the presence of increasing amounts of Con A (Fig. 3). Swainsonine-treated cells responded maximally at a lower concentration of Con A and incorporated approximately twice as much [^{3}H]thymidine as untreated cells. Castanospermine-treated cells also showed an enhanced sensitivity to Con A stimulation and enhanced thymidine incorporation, although the response was considerably lower than that with swainsonine. The substantial response of castanospermine-treated cells to Con A stimulation demonstrates that the inhibition observed in antigen-specific proliferation is not simply due to toxic effects or nonspecific inhibition of proliferation or DNA synthesis. However, if increased numbers of Con A-binding sites were the only contributor to enhanced Con A stimulation, one would expect that proliferation of castanospermine-treated cells should equal or exceed proliferation of swainsonine-treated cells. This was not observed. In fact, the inhibitory effect of castanospermine does not appear to be related to alteration of cell-surface carbohydrate involved in the cell-triggering step.

Secreted Amounts of T-Cell Growth Factor Are Not Altered by Pretreatment with Inhibitors; However, the Proliferative Response to Exogenous Factor Is Substantially Decreased by Both Inhibitors. Treated and control clone 46 cells were stimulated with irradiated spleen cells for either 6 or 24 hr. At each time, the culture supernatants were harvested and assayed for IL-2 content by titrating their ability to stimulate the proliferation of the IL-2-dependent cell line CTLL2. No significant difference in proliferation was observed at any dilution of the supernatants as compared to controls (data not shown). The indicator cell line used is also responsive to interleukin 4 (IL-4; ref. 21). It is therefore possible that the proliferative response we measure is sensitive to the presence in the test supernatants of IL-4 in addition to IL-2. However, the combined T-cell growth factor activity in the T-cell supernatants is not changed by inhibitor treatment. Deter-

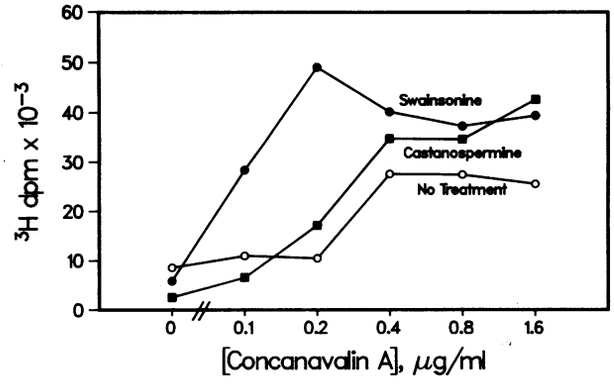

FIG. 3. Dose–response curve for proliferation to Con A as a mitogen. Clone 46 cells were incubated for 24 hr with medium, 1 μg of swainsonine per ml, or 100 μg of castanospermine per ml. The cells were harvested and distributed into microtiter wells (4×10^4) in the presence of the indicated concentration of Con A. Proliferation was determined by incorporation of a 6-hr pulse of 1 μCi of [^{3}H]thymidine at 72 hr. ○, Untreated; ●, swainsonine; ■, castanospermine.

mination of the possible contribution of IL-4 to the apparent IL-2 activity awaits further investigation.

To control for any direct effects of residual processing inhibitors on the assay, swainsonine or castanospermine was added directly to a control LCA supernatant, which was then titrated over the same range as the test supernatants. No effect of the added inhibitors on the IL-2 assay was observed. The lack of an effect on IL-2 production is consistent with the observation that added exogenous IL-2 did not overcome the stimulatory or inhibitory effects of the alkaloids on clone 46 proliferation (data not shown). In addition, no effect of inhibitor treatment was observed on the kinetics of overall protein synthesis, as determined by incorporation of [^{3}H]leucine (data not shown).

Cloned T cells are not truly resting T cells and express some residual IL-2 receptors on their surface 7 days after restimulation. The cells will therefore respond directly to exogenously added IL-2 in the absence of specific antigen stimulation. We analyzed the effect of swainsonine and castanospermine on the proliferative response to IL-2 by using as a source of the lymphokine a supernatant from phorbol ester-stimulated EL-4 tumor cells or LCA supernatant. The proliferative response to EL-4 supernatant was substantially reduced by swainsonine pretreatment of clone 46 cells (Fig. 4). Castanospermine-treated cells responded even less well to exogenous IL-2-containing supernatants. A greatly reduced response of swainsonine- and castanospermine-treated cells as compared to untreated cells was also observed with LCA supernatant as a source of IL-2 (data not shown). The possible contribution of IL-4 to T-cell stimulation by these supernatants has not been addressed.

Treatment with Swainsonine or Castanospermine Does Not Substantially Alter the Quantitative Expression of Cell-Surface Molecules Involved in T-Cell Responses. Stimulation of proliferation by antigen-bearing stimulator cells requires the function of several known T-cell surface glycoproteins. These include the multichain T-cell receptor/T3 complex and accessory molecules such as L3T4 and LFA-1 (22, 23). Enhanced expression of the high-affinity form of the IL-2 receptor and other growth factor receptors is also required (24, 25). A simple explanation of altered T-cell responses to antigen would be a quantitative change in the expression of the T-cell receptor complex. Since the helper T-cell clone L2 shows the same effects of inhibitors on antigen-specific proliferation as clone 46 and expresses a receptor reactive with the T-cell receptor-specific monoclonal antibody KJ16, we were able to ask whether overall expression of the receptor is altered by pretreatment with swainsonine or castanospermine. No substantial alteration in the level of receptor expression on the cell surface was detected by pretreatment with either processing inhibitor (Table 2). In addition, the levels of expression of the accessory molecule L3T4 or LFA-1 or the control molecule Thy-1 were not substantially altered on either clone 46 or clone L2. Since one obvious reason for the reduced responsiveness to exogenous IL-2 could be reduced expression of the IL-2 receptor, we also determined that the resting level of IL-2 receptor expression (as detected by antibody 7D4) following a 24-hr pretreatment with either inhibitor was essentially unchanged.

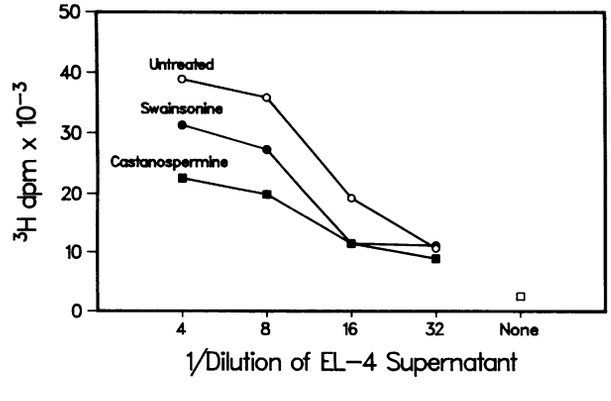

FIG. 4. Dose–response curve for proliferation to IL-2. Clone 46 cells were treated as in Fig. 3. The indicated dilution of EL-4 supernatant was added as a source of IL-2. Proliferation was measured as before. ○, Untreated; ●, swainsonine; ■, castanospermine; □, no supernatant.

Table 2. Antigen expression by treated T cells

T cell	Antigen*	Fluorescence intensity		
		Untreated	Swainsonine	Castanospermine
46	Thy-1	340	340	343
	IL-2 R	198	188	193
	L3T4	274	274	269
	LFA-1	292	288	281
L2	Thy-1	353	356	353
	IL-2 R	212	214	211
	TcR	200	209	202

Cells were incubated with inhibitors or medium for 24 hr and then stained with monoclonal antibodies of the indicated reactivities; this was followed by incubation with fluorescein-conjugated MAR18.5 and analysis on a FACS IV cell sorter. Staining with second antibody alone ranged from 124 to 141.

*R indicates receptor; TcR indicates the clone-specific T-cell receptor.

DISCUSSION

The results demonstrate that the glycosylation state of T-cell glycoproteins affects the ability of the cell to respond proliferatively to antigen or to other signals. The effect can be either stimulatory or inhibitory and depends on the nature of the carbohydrate alteration and of the proliferative stimulus. Although the proliferative response of the cells to antigen was affected by alkaloid pretreatment, the ability of the cells to receive an antigenic stimulus was not impaired. This was demonstrated by the lack of an effect on the production of IL-2, at 6 hr and at 24 hr after stimulation. The response to the signal for IL-2 production was the same in all cases. In these experiments, since we are using a T-cell clone, expansion of the IL-2-producing population was not required for IL-2 production. In contrast, in bulk spleen cell cultures where clonal expansion as well as IL-2 production must occur, the inhibitors might be expected to affect the quantity of IL-2 produced through indirect effects on proliferation. The net effect of altered proliferation could be enhancement or reduction of the overall immune response.

The stimulation of T-cell responses by swainsonine is in agreement with earlier work on inhibitor effects on immune responses. Hino *et al.* (26) demonstrated enhancement by swainsonine of lymphocyte *in vitro* proliferative responses to Con A. This was most pronounced in the presence of a suppressive factor isolated from tumor-bearing mice. The drug restored the suppressed mitogenic responses to control levels. Swainsonine also restored responses of MLCs containing the suppressive factor to control levels. The effects of swainsonine were also tested *in vivo* (27). Swainsonine treatment restored normal levels of anti-sheep erythrocyte antibody responses in animals immunosuppressed by tumor growth or by treatment with cyclophosphamide or mitomycin C. Swainsonine treatment also reduced tumor growth and metastasis *in vivo*. These results pointed to a stimulatory *in vivo* effect of swainsonine on the T-cell component of immune responses. The immunosuppressive effect of castanospermine as opposed to swainsonine is noteworthy in that a slight

alteration in carbohydrate structure causes a large change in T-cell function. Swainsonine and castanospermine have been considered as possible antiviral agents due to their effects on viral glycoprotein structure (6, 8, 9, 28). The elucidation of effects on immune function will be relevant to the evaluation of such compounds as therapeutic agents.

A variety of cell-surface N-linked glycoproteins have been characterized and shown to participate in the stimulation of T cells by antigen or other factors (22, 23). One or more of these molecules could be predominantly responsible for the alteration of T-cell responses. Our results indicate that the first part of activation—that is, triggering by antigen of IL-2 production—is unimpaired by alteration of the structure of N-linked glycoprotein oligosaccharides. At least for these T-cell clones, the altered T-cell receptor/T3 complex functions normally in triggering IL-2 production. However, processes in subsequent stages of activation are altered in a manner that either enhances or inhibits proliferation. One indicated alteration is in the ability of the IL-2 receptor to function, either in binding IL-2, in transmitting the binding signal, or in conversion of the low-affinity form to the high-affinity form containing both IL-2-binding subunits. Other processes must additionally be altered to give the differential effects seen in responses to antigen, mitogen, and IL-2. If the only effects were inhibition of growth factor receptor function, one would not expect to see the enhanced proliferation observed for swainsonine-treated cells. Continuing studies must focus on the function of the IL-2 receptor and assessment of the effects of the glycoprotein alterations on specific biochemical events that make up the activation pathway.

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1. Tkacz, J. S. & Lampen, J. O. (1975) *Biochem. Biophys. Res. Commun.* **65,** 248–257.
2. Kornbluth, J. (1985) *Cell. Immunol.* **95,** 276–287.
3. Wagh, P. V. & Bahl, O. P. (1981) *CRC Crit. Rev. Biochem.* **10,** 307–377.
4. Elbein, A D. (1984) *CRC Crit. Rev. Biochem.* **16,** 21–49.
5. Saul, R., Chambers, J. P., Molyneux, R. J. & Elbein, A. D. (1983) *Arch. Biochem. Biophys.* **221,** 593–597.
6. Pan, Y. T., Hori, H., Saul, R., Sanford, B. A., Molyneux, R. J. & Elbein, A. D. (1983) *Biochemistry* **22,** 3975–3985.
7. Elbein, A. D., Solf, R., Dorling, P. R. & Vosbeck, K. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 7393–7398.
8. Elbein, A. D., Dorling, P. R., Vosbeck, H. & Horisberger, M. (1982) *J. Biol. Chem.* **257,** 1573–1576.
9. Kang, M. S. & Elbein, A. D. (1983) *J. Virol.* **46,** 60–69.
10. Hohenschutz, L. D., Bell, E. A., Jewess, P. J., Leworthy, P. P., Pryce, R. J., Arnold, E. & Clardy, J. (1981) *Phytochemistry* **20,** 811–814.
11. Colegate, S. M., Dorling, P. R. & Huxtable, C. R. (1979) *Aust. J. Chem.* **32,** 2257–2264.
12. Glasebrook, A. L. & Fitch, F. W. (1980) *J. Exp. Med.* **151,** 876–895.
13. Wall, K. A., Lorber, M. I., Loken, M. R., McClatchey, S. & Fitch, F. W. (1983) *J. Immunol.* **131,** 1056–1064.
14. Malek, T. R., Robb, R. J. & Shevach, E. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5694–5698.
15. Dialynas, D. P., Quan, Z. S., Wall, K. A., Pierres, A., Quintans, J., Loken, M. R., Pierres, M. & Fitch, F. W. (1983) *J. Immunol.* **131,** 2445–2451.
16. Sarmiento, M., Dialynas, D. P., Lancki, D. W., Wall, K. A., Lorber, M. R., Loken, M. R. & Fitch, F. W. (1982) *Immunol. Rev.* **68,** 135–169.
17. Bekoff, M., Kubo, R. & Grey, H. M. (1986) *J. Immunol.* **137,** 1411–1419.
18. Gillis, S., Ferm, M. M., Lu, W. & Smith, K. A. (1978) *J. Immunol.* **120,** 2027–2031.
19. Lorber, M. I., Loken, M. R., Stall, A. M. & Fitch, F. W. (1982) *J. Immunol.* **128,** 2798–2803.
20. Cummings, R. D. & Kornfeld, S. (1982) *J. Biol. Chem.* **257,** 11235–11240.
21. Mosmann, T. R. & Coffman, R. L. (1987) *Immunol. Today* **8,** 223–227.
22. Oettgen, H. C. & Terhorst, C. (1987) *CRC Crit. Rev. Immunol.* **7,** 131–167.
23. Weiss, A., Imboden, J., Hardy, K., Manger, B., Terhorst, C. & Stobo, J. (1986) *Annu. Rev. Immunol.* **4,** 593–619.
24. Cantrell, D. A. & Smith, K. A. (1983) *J. Exp. Med.* **158,** 1895–1911.
25. Neckers, L. M., Yenokida, G. & James, S. P. (1984) *J. Immunol.* **133,** 2437–2441.
26. Hino, M., Nakayama, O., Tsurumi, Y., Adachi, K., Shibata, T., Terano, H., Kohsaka, M., Aoki, H. & Imanaka, H. (1985) *J. Antibiot.* **38,** 926–935.
27. Kino, T., Inamura, N., Nakahara, K., Kiyoto, S., Goto, T., Terano, H., Kohsaka, M., Aoki, H. & Imanaka, H. (1985) *J. Antibiot.* **38,** 936–940.
28. Walker, B. D., Kowalski, M., Goh, W. C., Kozarsky, K., Krieger, M., Rosen, C., Rohrschneider, L., Haseltine, W. A. & Sodroski, J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8120–8124.