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T-Cell-Dependent and T-Cell-Independent Mechanisms of Tolerance to Glucuronoxylomannan of *Cryptococcus neoformans* Serotype A

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Glucuronoxylomannan (GXM), a type 2 T-independent antigen, is the major component of the capsular polysaccharide (CnCAP) of *Cryptococcus neoformans*. Previous studies have described the tolerogenic effects of high doses of CnCAP on the specific humoral response. In this investigation, evidence for both high-dose and low-dose tolerance to GXM is presented. BALB/cBy female mice, primed with either 5 ng or 50 μg of GXM, then coimmunized 3 days later with immunogenic doses of both GXM and type 3 pneumococcal polysaccharide (SSS-III), showed an antigen-specific inhibition in their splenic plaque-forming cell (PFC) responses to GXM compared with control groups primed with normal saline. SSS-III PFCs remained unchanged between GXM-primed and normal saline-primed groups. Low-dose tolerance appeared to be T dependent, whereas high-dose tolerance appeared to be T independent. Low-dose tolerance to GXM could not be induced in athymic BALB/c *nu/nu* mice, whereas high-dose tolerance in the same mice could be induced. Furthermore, low-dose tolerance was adoptively transferred with B-cell-depleted splenocytes to naive BALB/c mice, while high-dose tolerance was not. Complement-mediated depletion of $CD4^+$ but not $CD8^+$ splenocytes from low-dose-primed mice abrogated the transfer of low-dose tolerance. These findings indicate T-dependent and T-independent mechanisms of antigen-specific B-cell tolerance to GXM in BALB/c mice at low and high antigen doses, respectively.

The capsular polysaccharide (CnCAP) of *Cryptococcus neoformans* is composed of three distinct antigens: a major antigen, glucuronoxylomannan (GXM) (11), and two minor antigens, a galactoxylomannan (14) and a mannoprotein (29). The tolerogenicity of CnCAP has been well documented (21, 25). However, most of the studies have only reported on the phenomenon of high-dose tolerance to preparations of CnCAP containing all three antigens. The proposed mechanism of this tolerance is that high levels of antigen work directly and reversibly to induce clonal B-cell anergy (21). However, the complexity of CnCAP has hindered research of antigenic specificity, and T-dependent (T_{DEP}) mechanisms that may also be involved have not been ruled out. Furthermore, low-dose tolerance, as reported for pneumococcal polysaccharide (SSS-III) (6), has not been studied. We have recently described the type 2 immunological properties of the purified major polysaccharide antigen of CnCAP, GXM (26). In this investigation, we examined B-cell tolerance to GXM relative to SSS-III to answer the following questions: (i) can tolerance to GXM be induced at both high and low antigen doses, and (ii) if so, is tolerance mediated by T_{DEP} or T-independent (T_{IND}) pathways, and (iii) if T_{DEP} mechanisms are involved, can the cell surface phenotype of the T cells responsible be identified? Antigen-specific tolerance to both high (50 μg) and low (5 ng) doses of GXM was observed at the plaque-forming cell (PFC) level. T_{DEP} and T_{IND} mechanisms were identified by examining tolerance induction in nude mice and also by the adoptive transfer of antigen-specific tolerance to naive mice with splenic T lymphocytes from tolerized mice. High-dose tolerance appeared to be T_{IND}, whereas low-dose tolerance was shown to be T_{DEP} and mediated by $CD4^+$ T cells.

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MATERIALS AND METHODS

Mice. Adult female mice, 8 to 16 weeks of age and certified viral antigen free, were used exclusively. All inbred normal and mutant strains of mice were obtained from Jackson Laboratories (Bar Harbor, Maine). The normal inbred strain used was BALB/cBy. The athymic mutant strain used was BALB/cBy *nu/nu*. All mice were maintained at the research animal facility at Georgia State University. They were given food (Purina Lab Chow) and water ad libitum. During the experiments, all mice remained healthy and showed no overt signs of disease.

Antigens and immunizations. Native GXM, from a large-capsule variant of *C. neoformans* serotype A, strain CDC 9759, was isolated and purified by the method described by Cherniak et al. (11). SSS-III was a gift from Phillip J. Baker (National Institute of Allergy and Infectious Diseases). Tests for purity and working dilutions of these antigens have been described elsewhere (26, 28).

(i) Immunizations. All injections of antigen were made intraperitoneally. Antigens were diluted in normal saline (NS) so that the appropriate amount could be delivered in a total volume of 0.2 ml.

(ii) Adoptive transfer of splenocytes. Spleen cells from immunized BALB/cBy mice were collected, washed in Hanks' balanced saline solution (HBSS), and adjusted to a concentration of 10^8 cells per ml in pyrogen-free (USP grade) 0.15 M NaCl. A volume of 0.2 ml of the spleen cell suspension was then injected with a 27-gauge insulin syringe

into one of the lateral tail veins of each member of an experimental group of naive mice.

Hemolytic plaque assay and statistical methods. Direct splenic PFCs were detected as previously described (12, 15, 26). Experimental and control groups routinely consisted of five randomly selected, inbred mice of the same strain and age. Three spleen cell samples were measured from each mouse. The calculation of confidence intervals and levels of statistical significance between experimental and control groups by analysis of variance has been described previously (26).

Depletion of splenic B cells and T-cell subsets. The method for B-cell depletion has been described previously (5). Briefly, goat anti-mouse immunoglobulin G (IgG) (polyvalent) was made up to a concentration of 20 μg/ml in 0.5 M Tris buffer (pH 9.5) (coating buffer). Five milliliters of the coating buffer was then pipetted into 100-ml-diameter polystyrene petri dishes (Primaria). The dishes were left at 24°C for 1 h and then put at 4°C overnight. The coated plates were washed three times with 0.15 M phosphate-buffered saline (pH 7.2) just before they were used.

(i) B-cell depletion. Freshly teased spleen cells were washed once in HBSS and then resuspended in RPMI 1640 medium (GIBCO) to a final concentration of 10^7/ml. Ten milliliters of the cell suspension was added to each anti-Ig labeled plate. The splenocytes were incubated at 24°C for 30 min and then mixed with gentle swirling and allowed to incubate at 24°C for another 30 min. After 1 h, the nonadherent cells were collected, counted, and evaluated by flow microfluorometry (FMF).

(ii) Depletion of T-cell subsets. Complement-mediated antibody-dependent lysis of T-cell subsets was performed in the following manner. B-cell-depleted mouse splenocytes were washed once in HBSS and then made up to 10^7/ml in cytotoxicity medium (Cedarlane Laboratories). Then, an equal volume of either rabbit anti-mouse Thy-1 (Accurate Chemical Co.), diluted 1:40 per 10^7 splenocytes, or rat anti-mouse Lyt-2 (CD8) (ascites) or L3T4 (CD4) monoclonal antibody (MAb) (Sera-Lab), diluted 1:500 per 10^7 splenocytes, was added. The cells were incubated at 4°C for 1 h and then spun down and resuspended in their original volume of cytotoxicity medium with rabbit complement (Low-Teas M; Cedarlane Laboratories) diluted 1:10. After incubation at 37°C for 1 h, the depleted cells were washed again in HBSS and ready to use.

FMF analysis. Cell depletion was evaluated by FMF analysis. Specific B-cell depletion was measured by dual staining with phycoerythrin-labeled rat anti-mouse Thy-1.2 and fluorescein isothiocyanate (FITC)-labeled rat anti-mouse CD45R (B exon specific). Depletion of T-cell subsets was measured by dual labeling with phycoerythrin-labeled rat anti-mouse L3T4 (CD4) and fluorescein isothiocyanate-labeled rat anti-mouse Lyt-2 (CD8). All fluorochrome-labeled reagents were purchased from PharMingen (San Diego, Calif.). Samples consisting of 10^6 stained splenocytes were subjected to FMF analysis with a FACS-STAR (Becton Dickinson Immunocytometry Systems). A total of 10,000 gated live lymphocytes were measured. Statistical analysis of the distribution of each ligand was determined by BD Consort 32 data-processing software.

The number of lymphocytes in individual T-cell subsets was reduced to less than 1% of the total population of panned nonadherent splenocytes with the specific MAbs and complement-mediated lysis. The number of B cells was depleted to within 8% of the total population of panned splenocytes.

TABLE 1. Absence of antigenic competition between GXM and SSS-III in BALB/c mice

Antigen	Immunization with single antigen[a]		Coimmunization with both antigens[a]	
	Geometric mean PFC per spleen ± CI[b]	Back mean[c]	Mean PFC per spleen ± CI[d]	Back mean
SSS-III	9.16 ± 0.50	9,463	8.96 ± 0.28	7,817
GXM	8.16 ± 0.40	3,490	7.71 ± 0.20	2,225

[a] No significant difference between experimental groups; analysis of variance F statistic = $F_{(2)1,8}$.
[b] CI, confidence interval.
[c] Anti-ln of geometric mean PFC counts.
[d] Geometric mean number of PFC per spleen with confidence intervals.

Because the fluorescein isothiocyanate-labeled anti-CD45 MAb used detects all B cells including pre-B cells that do not have membrane-bound Ig, we evaluated B-cell depletion by two other methods. First, we found that more than 95% of all nonadherent splenocytes were susceptible to lysis by anti-Thy-1.2 antibody and complement, as measured by trypan blue dye exclusion. Second, the PFC responses of BALB/c mice given immunogenic doses of SSS-III or GXM could be completely eliminated by panning the splenocytes on anti-Ig-coated plates.

RESULTS

Induction of antigen-specific tolerance. Induction of tolerance involves priming with a tolerogenic dose of the antigen and then immunizing with an immunogenic dose of the same antigen. Tolerance was observed by a significant reduction in PFCs compared with NS-primed controls. To determine the specificity of tolerance to GXM, we coimmunized groups of BALB/c mice with immunogenic doses (0.5 μg) of both GXM and SSS-III after priming with either NS or tolerogenic doses of GXM (5 ng or 50 μg). The antigenic specificity of tolerance to GXM was then measured by comparing anti-GXM and anti-SSS-III PFC responses of GXM-primed groups to those of NS-primed control groups. Control experiments, comparing the PFC responses of mice coimmunized with GXM and SSS-III to the PFC responses of groups immunized with either GXM or SSS-III alone, revealed no significant antigenic competition or cross-reactivity between the two antigens (Table 1).

Induction of low-dose tolerance to GXM. Although high-dose tolerance to cryptococcal antigens has been reported (9, 17, 22), low-dose tolerance to polysaccharides of *C. neoformans* has not. Having determined the type 2 T_{IND} characteristics of GXM (26), we chose to investigate tolerogenicity at low doses, as has been reported for another type 2 T_{IND} antigen, SSS-III (3). We primed two groups of mice with either NS or a low dose (5 ng) of GXM. Five days later, both groups were coimmunized with immunogenic doses (0.5 μg) of GXM and SSS-III, and the corresponding PFC responses were measured 5 days postimmunization. Low-dose priming with GXM caused a significant reduction ($P < 0.05$) in the PFC response to GXM but not to SSS-III (Table 2).

We next investigated T-cell involvement in low-dose tolerance to GXM. Two groups of athymic BALB/c *nu/nu* and ?/+ controls were primed with 5 ng of either GXM or NS. The mice were then immunized 5 days later with 0.5 μg of GXM, and PFC responses were measured 5 days postimmu-

TABLE 2. Induction of antigen-specific low-dose tolerance with 0.005 μg of GXM in athymic versus euthymic BALB/c mice

Mouse strain	Antigen (0.5 μg, 5 days)	GXM primed		NS primed		P_{H_0}[a]
		Geometric mean PFC per spleen ± CI[b]	Back mean[c]	Geometric mean PFC per spleen ± CI	Back mean	
BALB/cBy	SSS-III	10.65 ± 0.03	42,156	10.61 ± 0.14	40,625	NS[d]
BALB/cBy	GXM	7.40 ± 0.44	1,631	8.83 ± 0.10	6,812	$0.005 < P < 0.001$
BALB/cBy *nu/nu*	GXM	7.73 ± 0.31	2,280	8.07 ± 0.19	3,190	NS
BALB/cBy ?/+	GXM	6.84 ± 0.65	938	8.85 ± 0.37	7,008	$0.01 < P < 0.005$

[a] Probability that the null hypothesis, H_0 (that there is no difference between experimental groups), is true as measured by the statistic $F_{(2)1,8}$.
[b] CI, confidence interval.
[c] Anti-ln of geometric mean PFC counts.
[d] NS, no significant difference between experimental groups.

nization. Low-dose tolerance to GXM could not be induced in the athymic mice but could be induced in the wild-type control mice (Table 2).

Induction of high-dose tolerance to GXM. We investigated high-dose tolerance to GXM in a similar manner. Two groups of BALB/c mice were primed with either NS or a high dose (50 μg) of GXM. As described above, the mice were coimmunized 5 days later with 0.5 μg of GXM and SSS-III, and then antigen-specific PFC responses were determined 5 days postimmunization. Again, PFC responses to GXM were significantly reduced ($P < 0.001$) by priming with 50 μg of GXM but not by priming with NS, while PFC responses to SSS-III were statistically the same between both groups (Table 3). However, when we examined the effects of priming with high doses of GXM on BALB/c nude mice, we observed a different phenomenon. Both athymic and ?/+ control mice were able to be tolerized by priming with 50 μg of GXM (Table 3). These results suggest two different mechanisms of tolerance induction to GXM: one which requires an intact thymus for low-dose tolerance and one which is thymus-independent high-dose tolerance.

Adoptive transfer of antigen-specific tolerance to GXM. To test the hypothesis that tolerance to GXM could be induced by T_{DEP} or T_{IND} mechanisms, we conducted a series of experiments to see whether antigen-specific tolerance could be transferred to naive mice with B-cell-depleted splenocytes. Spleen cells from a group of antigen-primed mice were pooled and panned as described in Materials and Methods. Twenty million splenocytes were then adoptively transferred into the lateral tail veins of naive mice which were coimmunized with 0.5 μg of GXM and 0.5 μg of SSS-III at the same time. The antigen-specific PFC responses of the mice were measured 5 days postimmunization.

(i) Adoptive transfer of antigen-specific high-dose tolerance. We corroborated the T_{IND} pathway of high-dose tolerance to GXM by conducting a set of adoptive transfer experiments as described in the previous section. B-cell-depleted splenocytes from mice primed with NS or with 50 μg of GXM were adoptively transferred to corresponding groups of naive mice. These mice along with an unprimed group were then challenged 5 days later with 0.5 μg of both GXM and SSS-III. The PFC responses for both GXM and SSS-III remained statistically unchanged among all three groups (Table 4). Thus, under the conditions used in this experiment, high-dose tolerance could not be adoptively transferred.

(ii) Adoptive transfer of antigen-specific low-dose tolerance. Two groups of BALB/c mice were primed with either NS or 5 ng of GXM. Five days later, their spleen cells were recovered, panned, and then adoptively transferred into two corresponding groups of naive mice. These two groups along with a third unprimed group were then immunized with immunogenic doses of GXM and SSS-III; their antigen-specific PFCs were then measured 5 days postimmunization. Adoptive transfer of B-cell-depleted spleen cells from low-dose-primed mice reduced ($P < 0.15$) the GXM-specific PFC response. The PFC response to GXM was the same in the unprimed group and the group receiving NS-primed and panned splenocytes. Likewise, there was no statistical difference in the PFC responses to SSS-III in all three groups (Table 5).

We next conducted a series of experiments to study the level of reduction of PFC responses to GXM when B-cell-depleted splenocytes were transferred 12, 24, 72, and 120 h after low-dose priming. We found that adoptive transfer of B-cell-depleted splenocytes 12 to 24 h after low-dose priming improved the tolerogenic effect ($P < 0.05$, Table 6).

Adoptive transfer of low-dose tolerance with CD4$^+$ (Lyt2$^+$)

TABLE 3. Induction of antigen-specific high-dose tolerance with 50 μg of GXM in athymic and euthymic BALB/cBy mice

Mouse strain	Antigen	GXM primed		NS primed		P_{H_0}[a]
		Geometric mean PFC per spleen ± CI[b]	Back mean[c]	Geometric mean PFC per spleen ± CI	Back mean	
BALB/cBy	SSS-III	9.91 ± 0.18	20,125	9.69 ± 0.23	16,125	NS[d]
BALB/cBy	GXM	4.14 ± 0.71	1	8.83 ± 0.10	6,812	<0.001
BALB/cBy *nu/nu*	GXM	0.09 ± 1.4	0	8.52 ± 0.21	5,013	<0.001
BALB/cBy ?/+	GXM	2.06 ± 0.58	7.9	8.13 ± 0.25	3,397	<0.001

[a] Probability that the null hypothesis, H_0 (that there is no difference between experimental groups), is true as measured by the statistic $F_{(2)1,8}$.
[b] CI, confidence interval.
[c] Anti-ln of geometric mean PFC counts.
[d] NS, no significant difference between experimental groups.

TABLE 4. Adoptive transfer of antigen-specific high-dose tolerance to GXM

Spleen cells transferred	PFC response to GXM		PFC response to SSS-III	
	Geometric mean PFC per spleen ± CI[a]	Back mean[b]	Geometric mean PFC per spleen ± CI	Back mean
GXM primed	9.58 ± 0.42	14,419	10.75 ± 0.32	46,518
NS primed	9.27 ± 0.42	10,572	10.94 ± 0.40	56,110
None	9.17 ± 0.69	9,624	10.67 ± 0.39	42,860

[a] CI, confidence interval.
[b] Anti-ln of geometric mean PFC counts.

but not CD8$^+$ (L3T4$^+$) spleen cells. It has been previously reported that tolerance to another type 2 T_{IND} antigen, SSS-III, can be adoptively transferred by CD8$^+$ splenocytes from BALB/c mice that have been low-dose primed (5 ng) to naive BALB/c mice (27). We therefore investigated the possibility that low-dose tolerance to GXM might also be mediated by CD8$^+$ T cells. One group of 10 mice was low-dose primed with GXM. Twenty-four hours later, their spleen cells were removed, pooled, and panned on anti-Ig-coated plates. The splenocytes were then divided into three groups. One group was treated with anti-CD8$^+$ MAb and complement, the second group was treated with anti-CD4$^+$ MAb and complement, and the third group was treated with complement alone. The complement-mediated depletion of the T-cell subsets was verified by FMF analysis as described in Materials and Methods. BALB/c mice in three groups of four received 2×10^7 splenocytes from each of the corresponding groups of complement-treated, GXM-primed splenocytes. At the same time mice in all three adoptive transfer groups plus a fourth control group were immunized with 0.5 μg of GXM, and GXM PFCs were measured 5 days postimmunization. Our results revealed that low-dose tolerance to GXM can be adoptively transferred by CD4$^+$ T cells and not by CD8$^+$ T cells. Furthermore, adoptive transfer of CD8$^+$ T cells from low-dose-primed mice causes a significant increase in the PFC response to GXM (Table 7).

DISCUSSION

The conditions which lead to induced B-cell unresponsiveness to a specific antigen include dose of the antigen, route of immunization, valence of the antigen, and the immunological maturity of the B cell at the time of exposure to the antigen (1, 13, 16). Tolerance results from clonal B-cell anergy or deletion. With T_{DEP} antigens, which require T-cell help to initiate a B-cell response, tolerance can be induced directly via specific suppressive effects elaborated by T cells or indirectly by the failure to receive the appropriate second signal from T helper cells following ligand interactions with the B-cell antigen receptor. On the other hand, T_{IND} antigens do not require T-cell help to initiate a specific proliferative response in B cells (10). It has been demonstrated that the binding of membrane-bound Ig antigen receptors to polyvalent type 2 T_{IND} antigens can lead to either B-cell proliferation or anergy depending on the degree of antigen receptor cross-linking, the antigen's epitope density, and the antigen's size (13). However, there is also growing evidence of T-cell involvement in modulating the response to type 2 T_{IND} antigens (24).

An important new finding from this study was that specific tolerance to the GXM of *C. neoformans* can be induced in BALB/c mice at both high and low doses at the level of the splenic antibody-forming cell. Although the tolerogenic effects of the CnCAP have been documented (21, 25), information on the specificity has been hindered by its antigenic heterogeneity. Furthermore, there are reports on the nonspecific immunosuppression that occurs in vivo during cryptococcosis (18) or in vitro with cryptococcal antigens (7). Antigen-specific tolerance is defined by a suppressed response to an immunogen following priming with a tolerogenic dose of the antigen, while intact immune responses to unrelated antigens are maintained. For this study, we chose SSS-III as the unrelated reference antigen. Both GXM and SSS-III are acidic capsular polysaccharides from microbial pathogens, and both are type 2 T_{IND} antigens with the same immunogenic dose in BALB/c mice (0.5 μg) (26). Since our data revealed that coimmunization of BALB/c mice with

TABLE 5. Adoptive transfer of antigen-specific low-dose tolerance to GXM

Spleen cells transferred	PFC response to GXM		PFC response to SSS-III	
	Geometric mean PFC per spleen ± CI[a]	Back mean[b]	Geometric mean PFC per spleen ± CI	Back mean
GXM primed	7.98 ± 2.19	2,923	11.05 ± 0.25	63,013
NS primed	9.64 ± 0.24	15,298	11.21 ± 0.25	74,234
None	9.53 ± 0.24	13,700	11.09 ± 0.32	65,808

[a] CI, confidence interval.
[b] Anti-ln of geometric mean PFC counts.

TABLE 6. Probability of adoptive transfer of low-dose tolerance to GXM versus time

Time (h) of adoptive transfer of splenocytes after low-dose priming[a]	Geometric mean no. of PFCs/spleen[b]	Back mean[c]	Probability (P) of no difference between GXM-primed and unprimed groups
120[d]	7.98 ± 2.19	2,923	<0.15
None[d,e]	9.53 ± 0.24	13,700	
72	7.97 ± 0.34	2,887	<0.10
24	7.70 ± 0.57	2,201	<0.05
12	7.28 ± 0.48	1,449	<0.01
None[e]	8.77 ± 0.38	6,426	

[a] Priming dose of GXM was 0.005 μg; immunizing dose at time of adoptive transfer was 0.5 μg.
[b] Geometric means are given ± their corresponding 95% confidence limits.
[c] Anti-ln of geometric mean PFC response.
[d] Group consisted of three mice.
[e] Control receiving only the immunogenic dose.

TABLE 7. Adoptive transfer of low-dose tolerance to GXM with CD4$^+$ versus CD8$^+$ T cells

Low-dose-primed spleen cells transferred	Geometric mean PFC response per spleen ± CI[a]	Back mean[b]	Statistical difference of group means from positive control group
CD8$^+$	9.64 ± 0.24	15,429	$P^c < 0.05$
CD4$^+$	8.29 ± 0.36	3,980	$P < 0.01$
CD8$^+$ + CD4$^+$	8.01 ± 0.78	3,153	$P < 0.01$
No transfer	9.14 ± 0.20	9,345	None

[a] CI, confidence limit.
[b] Anti-ln of geometric mean PFC response.
[c] Probability of no statistical difference between experimental group and control group.

GXM and SSS-III resulted in no appreciable antigenic competition (Table 1), we were able to determine that antigen-specific tolerance to GXM occurs at both high-dose (Table 3) and low-dose (Table 2) levels.

A second new finding of this research was that antigen-specific high- and low-dose tolerance to GXM occurs via T_{IND} and T_{DEP} mechanisms, respectively. Although the antigenic specificity was not clearly defined, early studies by Kozel et al. (17) and Murphy and Cozad (22) demonstrated that exposure to high doses of CnCAP (100 to 800 μg) resulted in a transient tolerance in mice that could be measured in both splenic PFC responses and in serum antibody titers. Furthermore, Kozel et al. (17) reported that the maintenance of tolerance seemed to be linked to the persistence of detectable antigen in serum and tissues. These findings supported the idea that antigen-mediated mechanisms were the pathways by which the cryptococcal polysaccharide antigens inhibited the immune response. However, Baker's (2) work with SSS-III showed that low doses of the polysaccharide could induce tolerance that was mediated by T cells. That is, low-dose tolerance to SSS-III could be adoptively transferred from tolerized euthymic mice to naive mice with $CD8^+$ T cells (2).

When we examined high-dose tolerance to GXM, we found that tolerance could be induced in athymic mice (Table 3) but that it could not be transferred with T cells from high-dose-primed mice to naive mice (Table 4). These data support findings of Murphy and Cozad (22), Kozel et al. (17), and Breen et al. (9) and indicate that high-dose tolerance to the polysaccharide occurs by a T_{IND} mechanism. Furthermore, the fact that insignificant numbers of antibody-forming cells are detected following immunization with 50 μg of GXM rules out antibody feedback mechanisms of tolerance. Thus, it appears likely that direct interaction of B cells with the GXM results in anergy of antigen-specific splenocytes.

Alternately, low-dose tolerance to GXM appeared to be T_{DEP}, which agrees with the findings of Baker et al. (4) with another type 2 T_{IND} antigen, SSS-III. Low-dose tolerance to GXM could not be induced in nude mice (Table 2) but could be adoptively transferred to naive mice with T cells (Table 5). Furthermore, after immunization with 5 ng of GXM, neither antigen nor antibody are present in sufficient amounts (26) to account for antigen-mediated or antibody-mediated mechanisms for the GXM-specific tolerance that was detected. Thus, concluding that low-dose tolerance to GXM is mediated by T cells, we next examined the cell surface antigen phenotype of the T cells responsible for the tolerance induction.

The third important observation in this study was that adoptive transfer of $CD4^+$ cells from low-dose-primed mice to naive mice transferred tolerance to GXM, while adoptive transfer of $CD8^+$ cells enhanced ($P < 0.05$) the PFC response beyond the control group (Table 7). This result was unexpected since Baker (2) had reported that low-dose tolerance to SSS-III induced in BALB/c mice could be adoptively transferred with $CD8^+$ T cells to naive mice. Nevertheless, new evidence supports the belief that B-cell responses to type 2 T_{IND} antigens are regulated by $CD4^+$ T cells (23). Suppression of B-cell responses to type 2 T_{IND} antigens has been shown to be mediated by gamma interferon (19). Also, Van Den Eertwegh et al. (30) have shown that the type 2 T_{IND} antigen trinitrophenyl-Ficoll will induce the production of gamma interferon in $CD4^+$ cells. It is therefore tempting to speculate that low-dose priming with GXM induces $CD4^+$ TH1 cells which secrete gamma interferon (20) that mediates the suppression. Cytokine patterns may also explain the enhanced PFC response found with the adoptive transfer of $CD8^+$ cells (Table 7). In a recent review article, Bloom et al. (8) describe type 1 and type 2 classes of $CD8^+$ cells that elaborate patterns of cytokines similar to $CD4^+$ TH1 and TH2. It may be that the enhancement seen with the adoptive transfer of $CD8^+$ T cells from the low-dose-primed group may be due to a combination of the activation of type 2 $CD8^+$ and the depletion of $CD4^+$ type 1 cells. These speculations will need to clarified by future studies on patterns of cytokines produced by splenocytes from mice primed with low doses of GXM. Also, it is not completely clear how cytokines alone can account for the specificity of tolerance to GXM; anti-idiotypic mechanisms may also be involved.

In summary, we present evidence for antigen-specific B-cell tolerance to both high (50 μg) and low (5 ng) doses of GXM in BALB/c mice. Induction of tolerance is T-cell independent at the high dose and is T-cell dependent at the low dose. Furthermore, low-dose tolerance can be adoptively transferred with $CD4^+$ T cells.

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