ISOTYPE-SPECIFIC IMMUNOREGULATION

Evidence For A Distinct Subset Of T Contrasuppressor Cells for IgA Responses in Murine Peyer's Patches

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The mucosal surfaces of the gastrointestinal tract are continually exposed to antigens from the environment, and specific immunologic responses are induced for protection of the host. Orally encountered antigens are selectively taken up by the gut-associated lymphoreticular tissue (GALT)[1] or Peyer's patches (PP), where all immunocompetent cells, including regulatory T cells, IgA-committed B cells, and accessory cells, necessary for the induction and regulation of IgA responses, are present (1–5). Oral administration of large doses of soluble proteins or continuous feeding of particulate, T cell–dependent (TD) antigens can induce systemic unresponsiveness to the antigen, an immunologic state termed oral tolerance (6). This lack of responsiveness has been attributed to immune complexes (7), antiidiotypic antibodies (8), or to Ts cells and their derived factors (4, 9–11). Most work to date suggests that Ts cells are induced in PP in response to excessive or prolonged antigenic stimulation, and their subsequent homing to peripheral lymphoid tissue, such as spleen, results in systemic unresponsiveness (4, 9, 10). Oral tolerance and secretory IgA responses have been shown to be simultaneous events, with oral administration of protein or bacterial antigen inducing both systemic unresponsiveness and salivary IgA antibodies (12).

Mice that are genetically resistant to bacterial LPS, e.g., the C3H/HeJ strain, exhibit an enhanced IgA response pattern to orally administered TD antigens, while normal, LPS-responsive C3H/HeN mice do not (13). Continued gastric intubation (GI) of LPS-responsive mice with SRBC results in the production of Ts cells and oral tolerance (14). Interestingly, oral tolerance to SRBC cannot be induced in C3H/HeJ mice, but instead, when these mice are given antigen

[1] *Abbreviations used in this paper:* FcR, Fc receptor; FcαR, Fcα receptor; GALT, gut-associated lymphoreticular tissue; GI, gastric intubation; IBFα, IgA-binding factor; PP, Peyer's patches; Tcs, T contrasuppressor cells; TD, T cell–dependent.

systemically after prolonged GI of SRBC, they elicit good secondary responses, largely of the IgA isotype (14, 15). From these results we initially proposed that endogenous gut LPS induces the generation of precursors of Ts cells in the PP of normal (LPS-responsive) mice, but not in the LPS-nonresponsive C3H/HeJ strain (15). Upon continuous exposure to antigen, these Ts cell precursors in C3H/HeN mice develop into mature Ts cells and migrate to peripheral lymphoid tissues where they mediate systemic unresponsiveness. An equally plausible explanation for the difference in oral tolerance induction in C3H/HeN and C3H/HeJ mice is that an enhanced contrasuppressor T cell (Tcs) circuit is present in C3H/HeJ PP and renders Th cells resistant to Ts cell influence, resulting in the elevated Th cell–mediated immune response that characterizes this mouse strain.

The Tcs cell circuit is composed of three different cell types, namely, Lyt-1$^-$, 2$^+$, I-J$^+$ inducer cells, Lyt-1$^+$, 2$^+$, I-J$^+$ transducer cells, and Lyt-1$^+$, 2$^-$, I-J$^+$ effector cells (16–20). Common characteristics of these Tcs cells include their adherence to *Vicia villosa* lectin and their expression of the membrane I-J determinant (16, 17). Molecular genetic studies (21) have raised questions about the precise nature of the I-J determinant, since I-J molecules were not found to be encoded in the I region of the H-2 gene complex on mouse chromosome 17. However, studies with allo-anti-I-J and anti-I-J mAbs (17, 18) have provided strong evidence that the I-J determinant is associated with Tcs cells. Tcs cells have been shown to prevent Ts cell–mediated suppression of Th cells in in vitro antibody responses (18, 19), and to convert tolerogenic signals to immunogenic ones in contact sensitivity (16, 20). In addition, we have recently shown (22) that splenic Tcs cells from C3H/HeJ mice, when adoptively transferred to C3H/HeN mice that were orally tolerized to SRBC, allow IgM, IgG, and IgA anti-SRBC PFC responses in recipient mice challenged with antigen.

Murine PP are enriched in T cell subsets with helper/amplifier and contrasuppressor cell activity (23). Green et al. (23) suggested that the Tcs cell circuit plays a role in allowing the induction of IgA responses in the presence of Ts cells that mediate systemic unresponsiveness. The existence of active contrasuppression in the spleens of C3H/HeJ mice has been previously established (22). In this study, we present evidence that PP of these mice also contain significant Tcs cell activity, and that the T cell subsets responsible for this contrasuppression are distinct from L3T4$^+$ (helper) and Lyt-2$^+$ (suppressor) T cells. Furthermore, we describe a Tcs cell subset that binds to an IgA-specific Th line and selectively promotes IgA responses when adoptively transferred into a tolerized host.

Materials and Methods

Mice.   C3H/HeJ (The Jackson Laboratory, Bar Harbor, ME) and C3H/HeN (National Institutes of Health, Bethesda, MD) mice were bred and maintained in laminar flow cabinets in a facility designed for immunocompromised mice. All mice used in these studies were 8–12 wk of age.

Oral Immunization and Systemic Challenge.   SRBC (Colorado Serum Co., Denver, CO) were washed extensively (eight times) in HBSS and resuspended to 50% in eight parts HBSS and two parts sodium bicarbonate (isotonic), as described previously (13–15). Mice were given 0.25 ml of this 50% suspension (~4 × 10^9 SRBC) by GI daily for 28 consecutive days. For peripheral immunization, a 20% SRBC suspension in HBSS was given intraperitoneally (0.1 ml).

Splenic and PP Cell Preparations.   Spleens and PP were aseptically removed and single-cell suspensions were prepared as previously described (5, 13, 24). Briefly, spleens were gently teased through sterile stainless steel screens into MEM (GIBCO, Grand Island, NY) supplemented with sodium bicarbonate, nonessential amino acids, sodium pyruvate, L-glutamine, and gentamicin (incomplete MEM; 13, 24). Cell debris and clumps were removed by gravity sedimentation, and the cell-enriched supernatant was removed, washed in incomplete MEM, and resuspended in appropriate medium. Carefully dissected PP were enzymatically digested in Joklik-modified MEM (GIBCO) containing Dispase® (5, 24). Cells that became dissociated were removed by pipetting, washed extensively in incomplete MEM, and resuspended in appropriate medium.

Purification of T Cells.   Splenic or PP cells were washed four to five times in incomplete MEM containing 10% FCS. After the final wash, cells were allowed to adhere to tissue culture plates. Nonadherent cells were treated with anti–mouse Ig and rabbit C, as described previously (13, 15). This procedure was repeated two more times, and the T cells were further enriched by separation on a Ficoll-Hypaque gradient. Cell purity was established using FITC-conjugated monoclonal anti-Thy-1.2 (Becton Dickinson & Co., Sunnyvale, CA) and tetramethyl rhodamine isothiocyanate–labeled goat anti–mouse Ig (κ + λ). The purified T cell populations consisted of 95–97% Thy-1.2$^+$ and <1% Ig$^+$ cells.

Tcs Cell Fractions.   *V. villosa* lectin (E.Y. Laboratories, Inc., San Mateo, CA) was dissolved in Tris buffer (0.05 M, pH 9.5) as a stock solution of 0.05 mg/ml and was stored at 4°C until use. A 5-ml solution was added to petri plates (100 × 15 mm; Falcon Labware, Oxnard, CA) and the plates were incubated at 25°C for 90 min, as described previously (16, 22). The plates were then washed three times with PBS and were incubated with PBS containing 2% FCS (15 min at 25°C). Splenic or PP T cells (10^7 cells/ml) in PBS plus 5% FCS were added to plates and incubated for 90 min at 4°C. After incubation, nonadherent T cells were removed, washed twice in medium, and retained for subsequent experiments. *V. villosa*–adherent T cells were eluted by addition of 5 ml of *N*-acetyl-D-galactosamine (1 mg/ml) in PBS with a 20 min (37°C) incubation. The *V. villosa*–adherent T cell fraction was washed twice with medium before use.

Purified T cell fractions were further treated with rat anti-Lyt-1 (Clone 53-7.313) or anti-Lyt-2 (Clone 53-6.72) mAbs for 30 min at 4°C, followed by incubation with anti–rat IgG and C for 30 min at 37°C. These treatments resulted in removal of >99% Lyt-1$^+$ or >98% Lyt-2$^+$ T cells, respectively, as determined by immunofluorescence staining with either FITC-labeled anti-Lyt-1 or -Lyt-2 antibodies. In other experiments, PP T cells were treated with anti-L3T4 mAbs (hybridoma GK1.5) and C (25). Additional T cell fractions were treated with anti-I-J^k (CL 8703; Cedarlane Laboratories, Hornby, Ontario, Canada) or monoclonal anti-I-J^k (4B11) and C.

PP Tcs Adherence to an Fcα Receptor (FcαR)$^+$ T Cell Line.   T-T hybridomas were made previously by fusion of the R1.1 T lymphoma cell line (Thy-1.2$^+$, TL$^+$, H-2^k, and FcαR$^-$) with a cloned PP Th cell that promotes IgA responses (PP Th A; Thy 1.2$^+$, Lyt-1$^+$, 2$^-$, H-2^k, and FcαR$^+$) (24, 26). We used a cell line designated Th HA$_1$ 9 (Thy-1.2$^+$, FcαR$^+$) in this study (26). Macroculture 24-well plates (Linbro Chemical Co., Hamden, CT) were incubated with 1 ml of 0.1% glutaraldehyde (in PBS) for 2 h at 25°C. After removal of the fluid, 1 ml of 5 × 10^6 Th HA$_1$ 9 cells was added to each well and the plate was spun at 1,000 rpm for 5 min. The supernatant was removed by quickly inverting the plate and 1 ml of 0.01% glutaraldehyde in PBS was added. After incubation for 30 min at 25°C, the wells were washed extensively with PBS and then were incubated with PBS-BSA (1%) for 1 h. After two additional washes, *V. villosa*–adherent PP Tcs cells were added and incubated for 1 h at 4°C. Nonadherent cells were collected, washed, and kept on ice until assayed. The Th HA$_1$ 9 adherent Tcs cell fraction was collected by vigorous pipetting and was further fractionated on Ficoll-Hypaque gradients to remove any residual Th HA$_1$ 9 cells before assay.

Adoptive Transfer.   Individual T cell fractions (1–5 × 10^4 cells) were adoptively transferred to orally tolerized C3H/HeN mice. 1 h later, mice were immunized intraperitoneally with 0.1 ml of a 20% suspension of SRBC. 4 d later, mice were killed, their spleens were removed, and we determined the number of anti-SRBC PFC present.

In Vitro Splenic Cultures. Spleen cell cultures were prepared from C3H/HeN mice previously rendered systemically unresponsive to SRBC by daily GI (see Oral Immunization and Systemic Challenge). Spleen cells were resuspended in incomplete MEM containing 2-ME (5×10^{-5} M) and 10% FCS (complete medium). The cells were cultured (5×10^6 cells/0.5 ml) in macroculture plates (Linbro Chemical Co.), with or without exogenous T cells, and were immunized with SRBC. In some experiments, PP single-cell suspensions were treated with a cocktail of mAbs (rat anti–mouse Thy 1.2, anti-Lyt-1, and anti-Lyt-2 antibodies) for 30 min at 4°C, followed by incubation with anti–rat IgG and C (30 min at 37°C) (24, 26). <0.1% of the resulting B cell population stained with FITC-anti-Thy-1.2, and cultures failed to elicit mitogenic responses to PHA or Con A, and failed to exhibit PFC responses to several TD antigens. These results showed that the B cell–enriched population was free of residual T cells. Th cell–enriched or –depleted fractions were added to B cell cultures and were immunized with SRBC. Cultures were incubated for 5 d at 37°C in an atmosphere of 7% O_2, 10% CO_2, and 83% N_2.

PFC Assay. Nonadherent cells were removed from in vitro cultures, washed, and resuspended in HBSS to an appropriate concentration for bioassay. For in vivo experiments, single-cell suspensions of murine splenocytes were washed and resuspended in HBSS at appropriate dilutions before the assay. Individual B cell cultures and spleen cell suspensions were assayed in triplicate for IgM, IgG, and IgA anti-SRBC PFC responses using the modified Jerne-Nordin slide method (5, 13, 24).

Statistics. The results of the in vivo and in vitro PFC responses were expressed as the mean ± SEM. The significance of the difference between means was determined by the Student's *t*-test.

Results

Characteristics of C3H/HeJ PP Tcs Cells That Reverse Oral Tolerance. Our past studies showed that continuous feeding of C3H/HeN mice with SRBC leads to systemic unresponsiveness, while this treatment in the C3H/HeJ strain results in anamnestic-type responses, largely of the IgA isotype (reference 14; Fig. 1 and Table I). The inability to induce tolerance in the C3H/HeJ strain was associated with enhanced levels of Th cells in PP and spleen, while C3H/HeN mice exhibited mainly Ts cell activity in these tissues (14, 15). We considered the possibility that C3H/HeJ mice may exhibit an enhanced contrasuppressor system in PP, which would account for this strain's elevated immune response pattern to orally administered antigens. To test this, purified PP T cells from C3H/HeJ mice orally immunized with SRBC were enriched by adherence to *V. villosa* lectin, and were adoptively transferred to orally tolerant, C3H/HeN mice. *V. villosa*–adherent T cells from PP allowed the development of IgM and IgG responses, and markedly increased IgA responses in tolerized C3H/HeN mice (Table I), a pattern similar to that seen in C3H/HeJ mice given SRBC orally and systemically challenged with this antigen. The nonadherent fraction, which was enriched in Th cells, did not reverse oral tolerance (Table I). Adoptive transfer of *V. villosa*–adherent or –nonadherent PP T cells from orally tolerized C3H/HeN mice into the C3H/HeN strain also did not reverse oral tolerance. This suggests that C3H/HeJ mice given SRBC orally develop a PP T cell subset that reverses oral tolerance when small numbers are adoptively transferred and shares the property of binding to *V. villosa* with previously described Tcs cells (16, 19, 22).

Since the C3H/HeJ strain exhibits significant Th cell activity to orally administered SRBC, it remained possible that a highly active Th cell subset binds to *V. villosa* and accounts for oral tolerance reversal. Clearly, the T cell subset responsible is Lyt-1$^+$, since enrichment of PP *V. villosa*–adherent cells by treat-
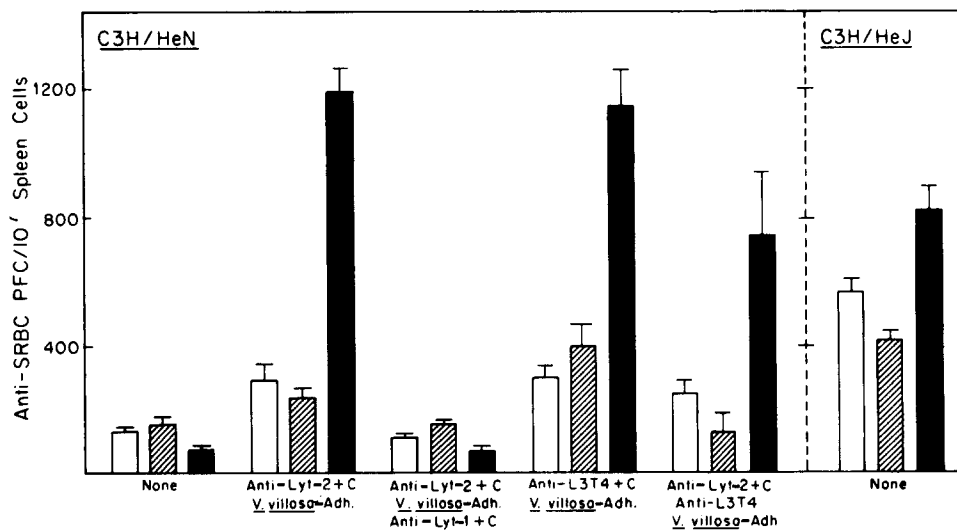
Adoptive Transfer of C3H/HeJ Peyer's Patch T Cells

FIGURE 1. Reversal of oral tolerance in C3H/HeN mice by adoptive transfer of C3H/HeJ PP T cells. PP T cells were obtained 7 d after a 4 wk schedule of daily GI with SRBC. T cells were treated with anti-Lyt-2 and/or anti-L3T4 and C followed by fractionation on V. villosa–coated plates. 10^4 adherent cells were adoptively transferred to tolerized C3H/HeN mice. In addition, anti-Lyt-2 and V. villosa–adherent T cells were treated with anti-Lyt-1 and C before transfer. Mice were challenged intraperitoneally with SRBC, splenic anti-SRBC PFC of the IgM (□), IgG (▨), and IgA (■) isotype were determined 4 d later. Adh, adherent.

TABLE I

Adoptive Transfer of V. villosa–adherent T Cells from PP of C3H/HeJ Mice Reverses Oral Tolerance in C3H/HeN Mice

Recipient	V. villosa lectin	Number of Cells transferred	Anti-SRBC PFC per 10^7 spleen cells*		
			IgM	IgG	IgA
C3H/HeN	—	None	130 ± 12	150 ± 21	68 ± 11
C3H/HeN	Adherent	5 × 10^4	206 ± 15	259 ± 76	667 ± 76
		1 × 10^4	292 ± 16	232 ± 10	781 ± 16
C3H/HeN	Nonadherent	5 × 10^4	107 ± 46	228 ± 43	137 ± 25
		1 × 10^4	139 ± 19	245 ± 11	110 ± 16
C3H/HeJ	—	None	568 ± 35	488 ± 31	824 ± 79

Groups of C3H/HeN and C3H/HeJ mice were given SRBC daily by GI for 28 d. 7 d later, PP cells from C3H/HeJ mice were enriched for T cells by removal of macrophages by adherence to plastic macroculture wells and by treatment with anti-Ig and rabbit complement. T cells were further fractionated by addition to V. villosa–coated plates, and nonadherent and adherent T cells were collected. Each fraction of T cells was adoptively transferred to orally tolerized C3H/HeN mice and mice were immunized intraperitoneally with SRBC. Splenic IgM, IgG, and IgA anti-SRBC responses were assessed on day 4.

* Values are the mean anti-SRBC PFC per 10^7 spleen cells ± SEM from triplicate slides and three separate experiments.

ment with anti-Lyt-2 and C gave a cell fraction that abrogated oral tolerance when adoptively transferred to C3H/HeN mice (Fig. 1). However, reversal was not due to mature Th cells, since treatment of PP T cells with anti-L3T4 mAb and C, followed by adherence to V. villosa, did not affect the ability of transferred T cells to abrogate tolerance (Fig. 1). In fact, this treatment removed the Th cell fraction, and addition of the resulting population to either splenic or PP B cell cultures immunized with SRBC did not produce a PFC response. Furthermore, treatment of Lyt-1$^+$, V. villosa–adherent PP T cells from C3H/HeJ mice with anti-L3T4 and C did not alter their ability to reverse oral tolerance in the C3H/HeN strain (Fig. 1). However, treatment of this fraction with anti-Lyt-1 and C completely removed this activity. We conclude from these results that C3H/HeJ mice given SRBC orally possess a PP Tcs cell subset that is Lyt-1$^+$, 2$^-$, V. villosa–adherent, and L3T4$^-$. It is suggested (16–20, 22) that contrasuppression in murine PP is due to a mature, effector Tcs cell population that is distinct from Th (L3T4$^+$) and Ts (Lyt-2$^+$).

Tcs cells share the important property of expression of the I-J determinant, and Tcs-derived factors that mediate contrasuppression also express this molecule (18, 27). When C3H/HeJ PP Lyt-1$^+$ T cell–enriched fractions were treated with either polyclonal anti-I-J^k or monoclonal anti-I-J^k antibodies and C, complete removal of Tcs activity was achieved (Fig. 2). Treatment of this T cell fraction with anti-I-J^d and C did not effect contrasuppressor activity. We conclude from these experiments that the cells that mediate reversal of oral tolerance in C3H/HeN mice are Lyt-1$^+$, 2$^-$, V. villosa–adherent, I-J^{k+} Tcs.

Characteristics of PP Tcs Cells That Convert Tolerant Splenic Cultures to Antibody Synthesis. It was important to establish that the reversal of oral tolerance is directly attributable to PP Tcs and not due to secondary effects resulting from in vivo cell interactions. Spleen cell cultures from C3H/HeN mice given SRBC orally for extended periods are unresponsive to this antigen in vitro. However, addition of 5×10^4 or 1×10^5 Lyt-1$^+$ T cells from C3H/HeJ PP converted tolerant splenic cultures to those supporting IgM, IgG, and IgA anti-SRBC PFC responses (Table II). We obtained higher in vitro PFC responses when we used the same numbers of Lyt-1$^+$, V. villosa–adherent T cells (Table II). The V. villosa–nonadherent fraction, on the other hand, was without effect. It should also be noted that IgA was the major isotype restored by Lyt-1$^+$, V. villosa–adherent Tcs cells from PP (Table II).

The PP T cell subset that converts splenic cultures from a tolerized to a responder state were shown to be Lyt-1$^+$ T cells, since prior treatment of PP T cells with anti-Lyt-2 and C did not affect contrasuppression (Table III). On the other hand, treatment of PP T cells with anti-Lyt-2 followed by anti-Lyt-1 and C completely removed their ability to support PFC responses in tolerized C3H/HeN splenic cultures (Table III). Again, the responsible Lyt-1$^+$ T cell was adherent to V. villosa lectin, suggesting that mature effector Tcs in PP were responsible for the observed conversion.

The Lyt-1$^+$, V. villosa–adherent PP T cell was shown to possess the I-J determinant, since treatment of Lyt-1$^+$ PP T cells with anti-I-J^k and C abolished contrasuppression in vitro (Table IV). From these results, we conclude that effector Tcs from C3H/HeJ PP are responsible for reversal of systemic unre-
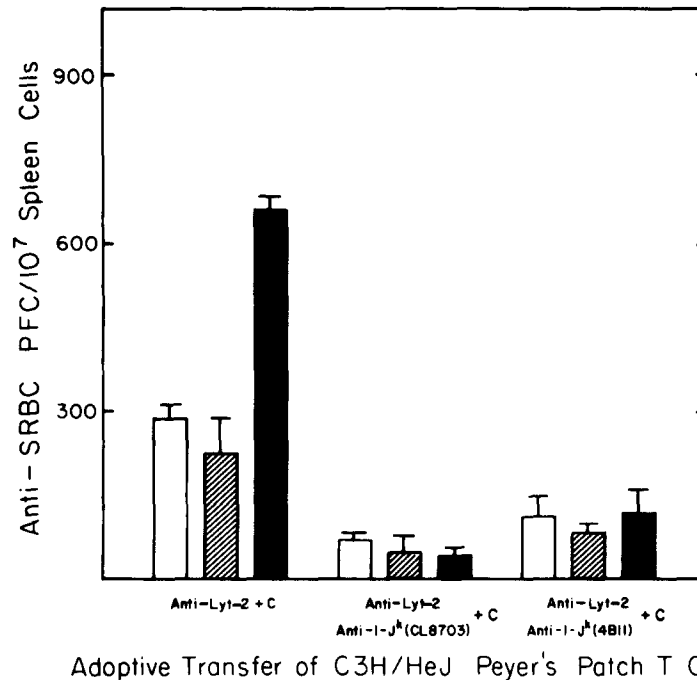
Adoptive Transfer of C3H/HeJ Peyer's Patch T Cells

FIGURE 2. Abrogation of PP Tcs cell activity by treatment with anti-I-J antibodies. C3H/HeJ PP T cells were treated with anti-Lyt-2 + C and additional aliquots were further treated with polyclonal anti-I-J^r (CL 8703) or monoclonal anti-I-J^r (4B11) and C. 10^4 T cells were transferred to orally tolerized C3H/HeN mice, the animals were immunized with SRBC, and splenic IgM (□), IgG (▨), and IgA (■) anti-SRBC PFC were determined 4 d later.

sponsiveness in C3H/HeN mice. Furthermore, when adoptively transferred into orally tolerized C3H/HeN mice, this Tcs subset mimics the response pattern seen in C3H/HeJ mice, suggesting that this active Tcs population may be responsible for the elevated IgA response pattern seen in C3H/HeJ mice orally immunized with TD antigens (13–15, 22).

C3H/HeJ PP Tcs Cells Potentiate IgA Responses. In a previous study (22), we also found that splenic Tcs from C3H/HeJ mice fed SRBC could reverse oral tolerance in C3H/HeN mice upon adoptive transfer. In these studies, good IgM, IgG, and IgA PFC were restored, and although IgA responses were highest, the other isotypes were also clearly enhanced (22). In contrast, adoptive transfer of Tcs from C3H/HeJ PP preferentially potentiate the IgA response (Table V). The adoptive transfer of splenic Tcs to tolerized C3H/HeN mice resulted in IgA PFC/IgG and IgM/PFC ratios (in the spleen) of 1.4 and 1.5. However, PP Tcs, when adoptively transferred, resulted in three to four times more IgA PFC than IgM or IgG PFC (Table V). This result suggested that splenic Tcs support all three isotypes equally well, while PP Tcs favor the IgA isotype. This could result from the presence of two (or more) effector Tcs subsets, one that supports IgM and IgG responses, and one that preferentially supports IgA responses.

Evidence for IgA-specific T Cell Networks. Our previous studies (28, 29) have shown that PP possess IgA-specific Th cells which collaborate with IgA-committed B cells through an apparent FcαR-mediated mechanism. We have established

TABLE II

V. villosa–adherent PP T Cells Abrogate the Unresponsiveness of Spleen Cells from Orally Tolerized Mice In Vitro

Cell treatment*	V. villosa*	Cell number added	Anti-SRBC PFC per culture		
			IgM	IgG	IgA
None	—	None	64 ± 12[‡]	29 ± 7	37 ± 7
Anti-Lyt-2 + C	—	1×10^5	81 ± 5	35 ± 13	230 ± 14
		5×10^4	110 ± 6	77 ± 9	207 ± 22
Anti-Lyt-2 + C	Adherent	1×10^5	173 ± 30	93 ± 20	302 ± 14
		5×10^4	165 ± 30	94 ± 21	391 ± 40
	Nonadherent	1×10^5	87 ± 10	47 ± 11	42 ± 13
		5×10^4	89 ± 10	26 ± 11	28 ± 17

Spleen cells (5×10^6) from C3H/HeN mice orally tolerized with SRBC were immunized with SRBC (2.5×10^6/culture) and were cultured for 5 d in the presence or absence of C3H/HeJ PP T cells (see below). IgM, IgG, and IgA anti-SRBC PFC responses were assessed on the final day of culture.
* PP from C3H/HeJ mice given SRBC by GI daily for 28 d were enriched for T cells by removal of macrophages by adherence to plastic culture plates and by treatment with anti-Ig and rabbit complement. T cells were further fractionated into a Lyt-1^{+} population by treatment with anti-Lyt-2 and C. Lyt-1^{+}-enriched T cells from C3H/HeJ PP were separated into adherent and nonadherent fractions on V. villosa lectin–coated plates.
[‡] Results are expressed as the mean anti-SRBC PFC per culture ± SEM from six cultures and from three separate experiments.

TABLE III

Contrasuppressor T Cells From PP Are Responsible for the Reversal of Splenic Unresponsiveness In Vitro

Treatment of Lyt-1^{+} T cells*	Cell number added	Anti-SRBC PFC per culture		
		IgM	IgG	IgA
None	None	55 ± 12[‡]	36 ± 6	36 ± 7
V. villosa–adherent	1×10^5	161 ± 23	91 ± 19	295 ± 13
	5×10^4	154 ± 25	85 ± 11	373 ± 46
V. villosa–adherent, anti-Lyt-1 + C	1×10^5	54 ± 4	73 ± 21	68 ± 15
	5×10^4	67 ± 10	57 ± 7	78 ± 17

Spleen cell cultures from C3H/HeN mice orally tolerized with SRBC were prepared as described in the Table II legend. IgM, IgG, and IgA anti-SRBC PFC repsonses were determined after 5 d of culture.
* Lyt-1^{+}-enriched and V. villosa–adherent T cells from C3H/HeJ PP were obtained as described in the Table II legend. Lyt-1^{+}, V. villosa–adherent T cells were treated with rat anti-Lyt-1 followed by anti–rat Ig and C.
[‡] Values are the mean anti-SRBC PFC per culture ± SEM from six cultures and from three separate experiments.

stable T cell lines from cloned PP Th cells that are FcαR[+] and that produce IgA-binding factor (IBFα), a molecule that regulates IgA responses in B cell cultures (26). If FcαR[+] PP Th cells are the major helper population involved in the IgA response, then one could postulate that isotype-specific Tcs potentiate IgA responses by an effect on this cell type. To test this assumption, C3H/HeJ PP T

TABLE IV

Anti-I-J^k and Complement Treatment Abrogates PP Contrasuppressor T Cell Activity In Vitro

Cell treatment*	Cell number added	Anti-SRBC PFC per culture		
		IgM	IgG	IgA
None	None	58 ± 10‡	38 ± 7	33 ± 6
Anti-Lyt-2 + C	1 × 10⁵	78 ± 8	46 ± 11	210 ± 17
	5 × 10⁴	102 ± 11	76 ± 16	287 ± 18
Anti-Lyt-2 + C,	1 × 10⁵	28 ± 5	21 ± 3	42 ± 3
Anti-I-J^k + C	5 × 10⁴	27 ± 7	30 ± 14	28 ± 10

Spleen cell cultures from C3H/HeN mice orally tolerized with SRBC were prepared as described in the legend for Table II. IgM, IgG, and IgA anti-SRBC PFC responses were determined after 5 d of culture.

* Lyt-1⁺-enriched T cells from PP of C3H/HeJ mice were prepared as described in the Table II legend. Lyt-1⁺ T cells were then treated with anti-I-J^k (CL 8703) and C.

‡ Results are expressed as the mean anti-SRBC PFC per culture ± SEM from six cultures and from three separate experiments.


TABLE V

Comparison of the Ability of Contrasuppressor T Cells from Spleen and PP of C3H/HeJ Mice To Abrogate Oral Tolerance in C3H/HeN mice

Cell source	Cell treatment	Anti-SRBC PFC per 10⁷ spleen cells*			Ratio of:	
		IgM	IgG	IgA	IgA/IgM	IgA/IgG
PP	Anti-Lyt-2 + C, V. villosa–adherent	267 ± 31	207 ± 23	895 ± 76	3.35	4.32
Spleen	Anti-Lyt-2 + C, V. villosa–adherent	454 ± 68	490 ± 48	675 ± 69	1.49	1.38
None	None	130 ± 12	150 ± 21	68 ± 11	—	—

PP or splenic T cells from C3H/HeJ mice given SRBC by GI for 28 d were prepared as described in the legend to Table I. T cells were further enriched for Lyt-1⁺ by treatment with rat anti-Lyt-2 followed by anti-rat Ig and C. Lyt-1⁺ T cells were added to V. villosa–coated plates and adherent fractions were adoptively transferred (5 × 10⁴ cells/mouse) to orally tolerized C3H/HeN mice, which were then immunized intraperitoneally with SRBC. IgM, IgG, and IgA anti-SRBC PFC responses were assessed 4 d later.

* Values are the mean anti-SRBC PFC per 10⁷ spleen cells ± SEM from triplicate slides and three separate experiments.

cells were enriched on V. villosa–coated plates and were further fractionated by adherence to solid-phase whole cell adsorbents of the Th HA$_1$ 9 cell line. PP T cells that failed to adhere to Fcα R⁺ Th HA cells, when adoptively transferred to C3H/HeN mice, supported only IgM and IgG anti-SRBC PFC responses (Fig. 3). On the other hand, V. villosa–adherent, Th HA–adherent Tcs cells supported all three isotypes when adoptively transferred (Fig. 3); however, the IgA response was clearly favored. PP Tcs cells were also added to R1.1 T lymphoma cells; however, no evidence for binding of specific Tcs subsets was seen. Since fractionation on solid-phase Th HA cells resulted in two populations of Tcs cells, an adherent Tcs cell subset that supported good IgA responses and a nonadherent
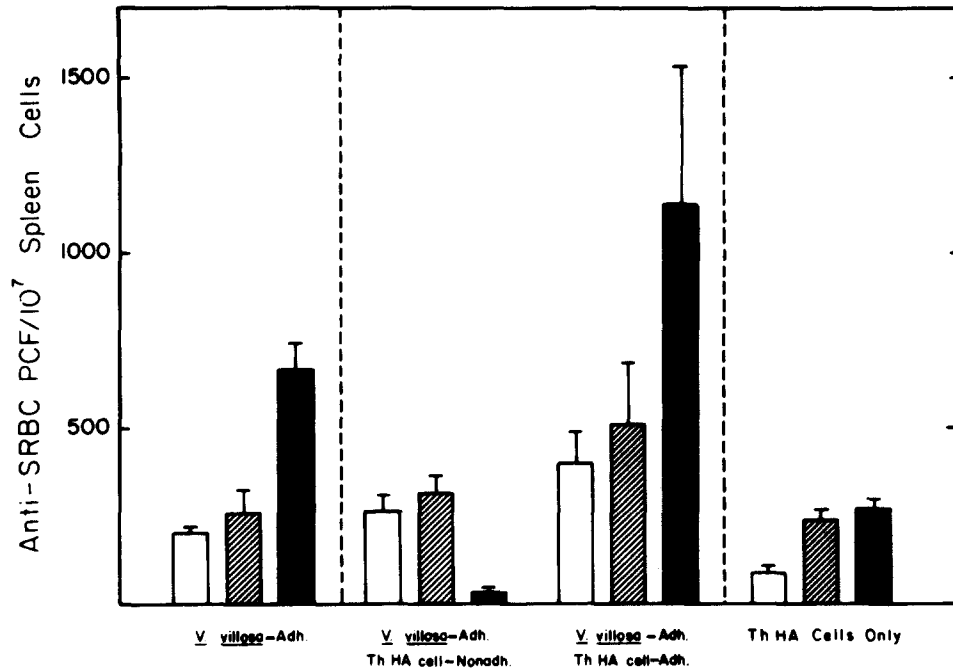
Adoptive Transfer of Peyer's Patch T Cells or Th HA Cells

FIGURE 3.   Evidence for IgA-specific Tcs cells. C3H/HeJ PP T cells were fractionated on *V. villosa*-coated plates and the adherent cells were removed and added to wells coated with Th HA$_1$ 9 cells. The Th HA cell–adherent and –nonadherent fractions were adoptively transferred (1 × 10^4 T cells) to C3H/HeN mice (see Fig. 1 legend for details). Mice were immunized intraperitoneally with SRBC, and splenic IgM (□), IgG (▨), and IgA (■) anti-SRBC PFC were determined 4 d later. Adh, adherent.

cell subset that did not, we conclude that Tcs cells can be IgA isotype-specific. Furthermore, IgA-specific Tcs cells are enriched at IgA inductive sites, e.g., the PP.

Discussion

The host normally encounters a myriad of antigens from the environment by ingestion, and the gastrointestinal tract maintains normal homeostasis through local IgA responses, and by systemic suppression of untoward responses to antigens that escape the gut and reach the internal immune system. This intricately balanced system must be tightly regulated, since it is dependent upon simultaneous induction of local IgA antibody responses and systemic unresponsiveness to the orally encountered antigen (oral tolerance). Considerable evidence now suggests that initiation of the local IgA response and systemic unresponsiveness both occur in GALT, e.g., PP. The simultaneous induction of Th cell pathways for an IgA response and Ts cell–mediated suppression have been described in murine PP after oral administration of protein (4, 12) or bacterial antigen (12); however, the precise mechanisms that allow both responses to occur have not been elucidated. The existence of a gut-associated contrasuppressor circuit of inducer, transducer, and effector Tcs cells that bear distinct I-J

determinants could subserve this role, since the Tcs effector cell blocks Ts cells and derived suppressor cell factors, and thus protects the Th cell (16–20, 22, 27). In this regard, murine PP contain significant Tcs cell activity (23). Differential homing of Ts cells from GALT to systemic lymphoid tissues (spleen) and of Th cells or IgA-committed B cells to mucosa would help explain how host responses occur; however, this does not address the mechanism for simultaneous induction of Th and Ts cells in GALT itself for maintenance of normal homeostasis.

The LPS-unresponsive C3H/HeJ mouse offered a unique opportunity to study GALT contrasuppression because this strain exhibits elevated Th activity in PP and enhanced IgA responses to orally administered TD antigens (13), and does not develop oral tolerance to SRBC (14, 15). The syngeneic, LPS-responsive C3H/HeN strain, however, is readily tolerized by feeding SRBC (14, 15). Adoptive transfer of small numbers of *V. villosa*–adherent T cells from C3H/HeJ PP reversed oral tolerance in the C3H/HeN mouse strain. This was not due to an active population of Th cells in this fraction, since treatment with monoclonal anti-L3T4 and C removed functional Th activity for anti-SRBC responses, but did not effect the adoptive transfer of responsiveness to SRBC in tolerized C3H/HeN mice. The active Tcs in C3H/HeJ PP exhibited the characteristics associated with contrasuppressor effector cells, and was $Lyt-1^+$, 2^-, $L3T4^-$, $I-J^{k+}$ and adherent to *V. villosa* lectin. This clearly suggests that the effector cell in the contrasuppressor T cell circuit is distinct from T cells that mediate help ($L3T4^+$) and suppression ($Lyt-2^+$). A more complete analysis of PP $L3T4^+$ Th and $Lyt-1^+$, 2^-, *V. villosa*–adherent, $L3T4^-$ Tcs cells is described elsewhere (K. Kitamura, H. Kiyono, J. H. Eldridge, D. R. Green, and J. R. McGhee, manuscript submitted for publication).

Several additional conclusions can be drawn from these observations. The fact that active Tcs effector cells from PP (this study) or spleen (22) of nontolerized C3H/HeJ mice convert tolerized C3H/HeN mice to responsiveness, clearly indicates that oral tolerance to SRBC is mediated by Ts cells. Since effector Tcs cells were adoptively transferred, it also suggests that Th cells are already present in tolerized mice, and are not induced after adoptive transfer of Tcs. Although this supposition has not been formally proven, it is supported by our previous demonstration that Th cells occur in spleen of orally tolerized C3H/HeN mice (14, 15). The absence of Th function when Tcs effector cells are not present, indicates that Ts cells override the helper activity and are responsible for systemic unresponsiveness. However, Tcs effector cells, together with Th cells, can overcome Ts-mediated tolerance and result in anamnestic-type responses to systemically administered antigen.

Studies with spleen cell cultures from tolerized C3H/HeN mice also suggest a central role for Tcs cells in reversing unresponsiveness. Addition of effector Tcs cells restored splenic cultures to a pattern of responsiveness that resembled that seen with adoptive transfer. Thus, Tcs cells can act directly upon splenic cell populations to reverse the tolerant state. The in vitro system used in this study should be amenable for use in studying the precise T cell–T cell interactions that occur in states of tolerance and antibody synthesis, including secondary-type responses. These studies are of importance, since we still have little understanding

of the function of Tcs and the cells with which they interact. In this regard, there is evidence (30, 31) that the OKT8$^+$ T cell population of humans, which mediates suppression, also contains a T cell subset that enhances OKT4$^+$ T cell help. The OKT8$^+$ T cell subset has the properties of binding to *V. villosa* lectin (32) and reacting with murine anti-I-J antibodies (33). Interestingly, the *V. villosa*–adherent, OKT8$^+$ T cell fraction binds to and presents antigen, and induces helper activity (32, 34). Effective antigen presentation to Th cells could thus represent an important Tcs cells function.

Perhaps the most striking observation resulting from these studies was the ability of C3H/HeJ PP Tcs cells to potentiate the IgA response. Other studies (22) had shown that splenic Tcs cells derived from C3H/HeJ mice fed SRBC also reverse oral tolerance when transferred to C3H/HeN mice. In those studies and the ones reported here (Table V), splenic Tcs cells supported IgM and secondary-type IgG and IgA responses. However, transfer of C3H/HeJ PP Tcs cells always resulted in three- to fourfold higher IgA responses than IgM or IgG responses. In addition, transfer of limiting numbers of splenic or PP Tcs cells gave higher ratios of IgA/IgM or IgA/IgG PFC, further suggesting that IgA-specific Tcs cells occur in enriched numbers in the GALT of C3H/HeJ mice (22 and data not shown).

Direct support for the existence of isotype-specific Tcs cells was provided by binding studies of PP *V. villosa*–adherent cells to an immobilized FcαR$^+$ Th line. Adsorption of Tcs to immobilized Th HA$_1$ 9 cells selectively depleted a cell population that could potentiate the IgA response. Interestingly, the nonadherent Tcs population maintained contrasuppressor cell activity, but only potentiated IgM and IgG anti-SRBC responses when adoptively transferred to tolerized C3H/HeN mice. Enrichment of IgA-specific C3H/HeJ PP Tcs cells by sequential adherence to *V. villosa* and Th HA$_1$ 9 cells gave a population that was active for enhancement of IgM and IgG responses, but that was most potent in enhancing IgA responses upon adoptive transfer (Fig. 3). Because of the low cell yields obtained by this procedure, it has been difficult to further characterize this Tcs cell fraction. We cannot at present discern whether one Tcs cell type is responsible for potentiation of all three isotypes with preference for IgA, or whether Tcs cells that support IgM and IgG responses contaminate the Tcs cell fraction that supports IgA responses. In our current studies, we are using various competitive inhibitors, such as purified IgA, monoclonal anti-FcαR, and antibodies to other T cell antigens, to selectively elute Th HA–bound Tcs cells. This should provide additional information regarding the mechanisms of Tcs–Th cell interactions and should allow further characterization of isotype-specific Tcs cells.

Our results provide a clearer picture of events that occur in GALT for induction of IgA responses and oral tolerance than has previously existed. Our past studies have shown that orally administered TD antigens induce the production of PP Th that preferentially support IgA responses (5, 13, 14, 24). Clones of PP Th bear FcαR and selectively collaborate with IgA-committed B cells for IgA synthesis (24, 28, 29). We know that the FcαR and secreted IBFα are important in this response, since FcαR$^+$ Th lines that constitutively produce IBFα support IgA responses to various TD antigens (26). The present study

suggests that effector Tcs cells in PP bind to $Fc\alpha R^+$ T cells and potentiate IgA responses in otherwise tolerant C3H/HeN mice. Thus, isotype-specific Tcs cells may protect $Fc\alpha R^+$ Th cells from suppressive signals in GALT for maintenance of IgA responses. These Tcs cells may not, on the other hand, prevent Ts cells induction at this site, which ultimately would lead to systemic unresponsiveness (oral tolerance). We still do not yet understand why LPS-nonresponsive C3H/HeJ mice exhibit an enhanced Tcs cell activity in response to orally administered SRBC. The Tcs cell circuit is regulated by $Lyt-1^+$, 2^+, $I-J^+$ Ts which give rise to level two suppression (17, 35), and it is possible that the GALT of C3H/HeJ mice is deficient in this regulatory T cell population. Regardless, this mouse strain continues to be useful for our dissection of the regulatory pathways that are involved in the induction of IgA responses, without the added complication of suppressor cell pathways in GALT.

We feel that it may be useful now to consider GALT as a unique immunologic organ as compared with systemic lymphoid tissues such as spleen. In this regard, one could propose that GALT contains two lymphoid cell compartments. The first (I) would consist of normal B cells (μ^+, δ^+) and regulatory T cells, e.g., Th, Ts, and Tcs, which either enhance or suppress IgM and IgG subclass responses. Compartment I cells, of course, would occur in other lymphoid tissues, and, for example, would represent the major cell types present in spleen. The second (II) compartment would be unique to GALT and would consist of a full repertoire of committed, $sIgA^+$ B cells, IgA-specific Th cells, and, as described in this study, a unique population of isotype-specific Tcs cells that act on $Fc\alpha R^+$ Th for potentiation of IgA responses. Oral administration of TD antigen would perturb both compartments, and compartment I responses would favor immunosuppression. Lymphocyte redistribution would ensure that Ts cells are present in peripheral lymphoid tissue for down-regulation of responses to antigens that escape from the gut tissues. Compartment II responses would involve only the IgA isotype and would result in the stimulation of IgA-committed B cells and the induction of $Fc\alpha R^+$ Th cells. The latter response would be under the full protection of IgA-specific Tcs cells present in GALT. It should be noted that selective homing of all compartment II cells to mucosal tissues, such as lamina propria regions of the gut, may occur; however, it would only be necessary to postulate that IgA-committed B cells are involved in this selective localization, a fact that is now well established (36, 37). Much remains to be learned about these two major pathways of host responses in GALT, and the precise regulatory processes that control each compartment. However, the availability of current molecular and cell biology systems should allow testing of these concepts and should ultimately lead to the elucidation of mechanisms involved in immunological homeostasis to antigens encountered from the gut environment.

Summary

The ability of murine Peyer's patch (PP) T contrasuppressor cells (Tcs) to reverse oral tolerance to the T cell–dependent (TD) antigen SRBC was studied both in vivo and in vitro. C3H/HeJ mice given SRBC orally for 4 wk are not rendered tolerant to this antigen and were used as a source of PP Tcs cells for adoptive transfer to identically treated, orally tolerized C3H/HeN mice. Transfer

of 10^4 or 5×10^4 *V. villosa*–adherent PP T cells resulted in splenic IgM, IgG, and mainly IgA responses in C3H/HeN mice challenged systemically with SRBC. The T cell responsible was Lyt-1^+, 2^-, L3T4$^-$, I-J$^{\kappa+}$ and *V. villosa* lectin–adherent, all characteristics of mature effector Tcs cells. This C3H/HeJ PP Tcs cell subset was also effective when added to in vitro cultures of tolerized spleen cells derived from SRBC-fed, C3H/HeN mice. Interestingly, C3H/HeJ PP Tcs cells restored mainly IgA responses when transferred in vivo or when added to suppressed C3H/HeN splenic cultures. Comparison of the functional activity of Tcs cells derived from spleen or PP of orally immunized C3H/HeJ mice revealed that splenic Tcs cells supported responses of all 3 isotypes; however, PP Tcs cells yielded three- fourfold higher IgA responses, when compared with IgM or IgG anti-SRBC responses. Adherence of C3H/HeJ PP Tcs to an FcαR$^+$ T cell line derived from IgA-specific Th cells resulted in a nonadherent cell fraction that potentiated only IgM and IgG responses, while bound Tcs cells preferentially supported IgA responses. These results suggest that murine PP contain IgA-specific Tcs cells that allow IgA response induction in the presence of Ts cells that mediate oral tolerance.

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