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T-cell lymphokine response to orally administered proteins during priming and unresponsiveness

G. F. HOYNE, M. G. CALLOW, J. KUHLMAN* & W. R. THOMAS *Division of Molecular Biology, Western Australian Research Institute for Child Health, Princess Margaret Hospital, Perth, Western Australia and *AMRAD Corporation Limited, Melbourne, Victoria, Australia*

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SUMMARY

Feeding antigens induces an immunological unresponsiveness termed oral tolerance but under some conditions, for example following the administration of cyclophosphamide (CY), immunity can be induced. These observations have usually been made by studying antibody production and delayed hypersensitivity with little attention given to other measurements of cellular activation. We have therefore examined the lymphokines produced by T cells obtained after the induction of oral tolerance or intragastric priming. Cells isolated from the spleen and Peyer's patches (PP) of tolerized mice could secrete high levels of interferon-γ (IFN-γ) and moderate levels of granulocyte–macrophage colony-stimulating factor (GM-CSF) in response to antigen while interleukin-2 (IL-2), IL-3 and IL-4 could not be detected. Mesenteric lymph node (MLN) cells of tolerized mice did not respond to antigen unless spleen adherent cells were added to the cultures where IFN-γ and GM-CSF were produced. Intragastric priming was achieved by feeding antigen to CY-treated mice. T cells from the spleen, MLN and PP of these mice could produce GM-CSF, IFN-γ, some IL-3 but little or no IL-2 and IL-4. The ability of MLN cells to proliferate with antigen *in vitro* was low and corresponded to low IL-2 production. Thus T cells from fed mice secrete a defined pattern of lymphokines which differs in tolerizing and priming regimes.

INTRODUCTION

Administering antigens by the intragastric (i.g.) route normally results in the development of an unresponsive state termed oral tolerance.[1–4] A similar phenomenon occurs in the respiratory tract following exposure to aerosolized protein[5] and evidence has been obtained in both systems to implicate the participation of suppressor T cells.[2,5] Experimentally, oestrogen[6] and the cytotoxic agent cyclophosphamide (CY) have been used to prevent the induction of oral tolerance and mice treated with these agents before feeding can develop delayed hypersensitivity and become primed for antibody production to the fed antigen.[7,8] Although some studies have examined migratory inhibitory factor (MIF) production, now known to be granulocyte–macrophage colony-stimulating factor (GM-CSF)[9] and intraepithelial lymphocyte (IEL) accumulation,[10] more direct measurements of T-cell responses have not been made. In this study we have therefore examined the interleukin response of T cells obtained from mice after feeding antigen using either (a) a regime known to produce oral tolerance, or (b) a regime known to produce sensitization that involved treating mice with CY 2 days prior to feeding. We show here that T cells from mice treated with CY and fed ovalbumin (OVA) secrete GM-CSF and interferon-γ (IFN-γ) after *in vitro* challenge, but unlike parenteral priming, do not produce IL-2. Surprisingly, feeding mice in a tolerizing regime sensitized T cells in the Peyer's patch (PP) and spleen which showed similar patterns of lymphokine release as those derived by CY priming (IL-3/GM-CSF, IFN-γ). Mesenteric lymph node (MLN) T cells from tolerized mice were unresponsive unless antigen was presented by splenic adherent cells.

Abbreviations: CY, cyclophosphamide; MLN, mesenteric lymph node; OVA, ovalbumin; PP, Peyer's patch.

Correspondence: Dr W. R. Thomas, Western Australian Research Institute for Child Health, Princess Margaret Hospital, GPO Box D184, Perth, Western Australia, 6001.

MATERIALS AND METHODS

Animals

BALB/c mice were provided by the Animal Resource Centre (Murdoch, Western Australia). All mice were females between the age of 6 and 8 weeks.

Feeding and immunization

Mice were given i.p. injections of CY (Asta, Brackwede, Germany) at doses indicated within the text. In general these ranged from 50 to 200 mg/kg body weight 2 days before feeding or immunization. Mice were lightly anaesthetized under ether

and fed i.g. by a tube. All mice received the appropriate concentration of antigen dissolved in phosphate-buffered saline (PBS) in a volume of 0·2 ml. To induce oral tolerance mice were fed on 3 consecutive days with 3 mg OVA (Sigma Chemical Co., St Louis, MO) and immunized after 7 days with 100 μg OVA in Freund's adjuvant (CFA; Difco, Detroit, MI). The periaortic and inguinal lymph node cells were collected 7 days later and cultured *in vitro* with OVA. Culture supernatants were then assayed for lymphokine activity. Subcutaneous immunization was given at the base of tail with 100 μg of antigen emulsified in CFA.

T-cell assays

Cell suspensions were prepared by expressing lymph node and PP cells through a stainless steel mesh. The cells were washed with Dulbecco's Modified Eagles Medium (DMEM; Gibco Laboratories, Grand Island, NY). To measure T-cell responses *in vitro* to OVA, lymph node cells were prepared as described above and cultured at 4×10^5 cells in 200 μl in DMEM supplemented with 2% foetal calf serum (FCS) (Commonwealth Serum Laboratories, Parkville, Victoria), 2 mM L-glutamine (Sigma), 50 μg/ml gentamycin and 50 μM 2-mercaptoethanol (2-ME) (Sigma). Antigen was then added at varying concentrations and the cells were cultured for 24 hr at 37° in 96-well flat-bottom tissue culture plates (Becton Dickinson Labware, Lincoln Park, NJ). The supernatants were then collected and stored at −20° until required. The proliferative response of i.g. primed lymph node cells was measured by culturing the cells *in vitro* for 4 days at 37° and pulsing them with 1 μCi [^{3}H]thymidine (Amersham, Australia, North Ryde, NSW) for a further 16 hr. The cells were harvested onto glass fibre filter mats (Enzo Diagnostics Inc., NY) and the amount of radioactivity incorporated was measured using liquid scintillation spectrometry.

Antigen presentation assays

Lymph node T cells were purified by incubating cell suspensions on a nylon wool column for 2 hr at 37°.[11] Splenic adherent cells were prepared by culturing spleen cells from naive mice for 2 hr at 37° on plastic tissue culture plates. Non-adherent cells were removed and the adherent cells carefully washed twice with warm DMEM. Ice-cold PBS containing 5 mM EDTA was then added and the cells incubated on ice for 15 min. Adherent cells were collected by vigorous pipetting and the cells were washed twice with normal medium before use. T cells were added to microtitre plates at 4×10^5 cells in a volume of 100 μl and splenic adherent cells were added at 8×10^4 in a 100-μl volume, antigen was added and the cells were incubated for 24 hr at 37°. The supernatants were then collected and stored at −20° until required.

Bulk lymphocyte culture

Bulk culture of lymph node cells was set up in 1 ml of culture medium/1 cm^2 tissue culture well (Costar, Cambridge, MA). Antigen was added at the indicated concentration and the cells cultured at 37° for 24 hr. Supernatants were collected and assayed for the release of lymphokines

Lymphokine assays of culture supernatants

Listed in parentheses are the lymphokine responsive or sensitive cell lines which were used to assess the bioactivities of the culture supernatants. These included IL-2 (CTLL-2), IL-3/GM-CSF (FDC-P1), IL-3 (32D), IL-4 (CT.4S) and IFN-γ 1WEHI-279.1). The methods used for each of the assays have been described in detail elsewhere.[12] A brief description of the methods and the specifities for each of the cell lines used is given. All lymphokine activities were assessed using a 1/2 dilution of supernatant in a total volume of 100 μl.

The CTLL-2 cell line proliferates maximally with IL-2 but only poorly in the presence of IL-4. Supernatants were cultured with 5×10^3 CTLL-2 cells/well for 24 hr at 37°.

The FDC-P1 cell line proliferates optimally in the presence of IL-3 or GM-CSF and weakly in the presence of IFN-γ and IL-4. Supernatants were incubated with 2×10^3 FDC-P1 cells for 48 hr at 27°.

The 32D cell line proliferates only in response to IL-3. Supernatants were incubated with 2×10^3 32D cells/well for 48 hr at 37°.

The CT.4S cell line proliferates maximally to IL-4 and only very minimally to IL-2. Supernatants were cultured with 2×10^3 CT.4S cells for 4 days at 37°.

The WEHI-279.1 cell line is a B-cell lymphoma whose growth is inhibited in the presence of IFN-γ.[13] Supernatants were incubated with 5×10^3 WEHI-279.1 cells for 3 days at 37°. To determine specifically for the presence of IFN-γ in culture supernatants a 1/20 dilution of anti-IFN-γ hybridoma HB170 supernatant was added to test samples. Those assays showing at least 90% neutralization of IFN activity were considered to contain IFN-γ.

The proliferative response of the individual cell lines was measured by the amount of [^{3}H]thymidine incorporated over a 6-hr pulse period using liquid scintillation spectrometry. Standards were included in all assays utilizing recombinant IL-2 and IL-4, WEHI-3B (D$^-$) conditioned medium as a source of IL-3 and concanavalin A (Con A)-activated T-cell supernatant as a source of IFN-γ. The FDC-P1, 32D, WEHI-279.1, CT.4S, WEHI-3B (D$^-$) and HB 170 cell lines were all provided by Dr A. Kelso (Walter and Eliza Hall Institute for Medical Research, Melbourne, Australia).

Statistics

Lymphokine responses for all groups are expressed as means ± 1 SD and all comparisons were made using a Student's *t*-test.

RESULTS

Lymphokine response of mice fed for oral tolerance

To induce and measure oral tolerance, mice were fed on 3 consecutive days with 3 mg OVA and immunized parenterally 7 days after the last feed with 100 μg OVA in CFA. The periaortic and inguinal lymph nodes were collected 7 days later and cell suspensions were examined for their ability to produce IL-3 GM-CSF after antigen stimulation *in vitro*. The results presented in Table 1 showed a profound unresponsiveness in the OVA-fed group in comparison to the control mice which had been fed saline. This result would indicate then that most, if not all OVA-reactive T cells become tolerized during this first week after feeding. Nevertheless, we were interested in determining the level of T-cell reactivity which remained in the gut associated lymphoid tissue (i.e. PP and MLN) and spleen following the induction of oral tolerance. Mice were fed a tolerizing dose (i.e.

Table 1. Oral tolerance measured by lymphokine release in mice fed OVA

Fed*	Saline	Ova	Ova
s.c. challenge	Ova	Ova	Saline
[OVA] (μg/ml)			
800	54,741 ± 2556†	4263 ± 1100	3968 ± 1150
400	35,076 ± 7550	3152 ± 1050	3773 ± 567
40	14,225 ± 1790	5290 ± 1525	2050 ± 208
4	10,087 ± 2345	2252 ± 750	748 ± 150

* Mice were fed saline or OVA on 3 consecutive days and 7 days later immunized with 100 μg of OVA in CFA. One week later the periaortic and inguinal lymph nodes were cultured *in vitro* with antigen and the supernatants assayed for IL-3/GM-CSF activity using the FDC-P1 line.

† Data represent the mean response of three mice and are expressed as d.p.m. ± SD. Statistical significance was calculated using the Student's *t*-test. Feeding OVA versus feeding saline, $P < 0{\cdot}01$.

Background responses for these assays were < 800 d.p.m.

three times with 3 mg OVA) and after 7 days (without any further immunization) cell suspensions were prepared from the PP, MLN and spleen, cultured *in vitro* with the antigen and after 24 hr the supernantants assayed for lymphokines. It was possible to discriminate between the release of IL-3 and GM-CSF in the culture supernatants by assessing the responses elicited by the 32D and FDC-P1 cell lines. The supernatants from the spleen and PP cells of tolerized mice could easily support the growth of the FDC-P1 cells (Fig. 1a) but not the IL-3 specific 32D cells (see legend to Fig. 1). This was taken to indicate that the major response measured by the FDC-P1 cells must be due to the release of GM-CSF. The culture supernatants obtained from tolerized mice were also found to contain large amounts of IFN-γ (Fig. 1a, b) but no IL-2 or 4 (data not shown, but all were negative in respect to the standards which gave the expected levels of stimulation within each assay). In contrast to the spleen and PP cells, no lymphokine responses could be detected in the MLN cells (Fig. 1a, b). However, if splenic adherent cells were cultured with purified MLN T cells in the presence of OVA then it was possible to detect the release of both GM-CSF and IFN-γ (Fig. 1c, d). Similarly, purified PP T cells cultured with splenic adherent cells produced higher levels of GM-CSF and IFN-γ than cultures of PP cells alone (compare Fig. 1a, b with Fig. 1c, d).

Lymphokine responses of T cell from mice immunized by i.g. administration of OVA following CY

BALB/c mice were injected with 0, 50, 100 or 200 mg/kg CY and 2 days later fed 750 μg OVA. The MLN cells were collected 8 days later and stimulated *in vitro* with OVA and 24-hr supernatants assayed for IL-3/GM-CSF (Fig. 2). Cells from mice not treated with CY did not produce a response but mice treated with CY gave large responses to OVA with the highest sensitization found with the highest dose of CY (200 mg/kg) (Fig. 2).

To determine what effect the quantity of fed protein would have on the lymphokine response after CY/OVA priming, mice were injected with 200 mg/kg CY 2 days prior to receiving 500, 750 or1000 μg OVA i.g. and the response of MLN cells was measured 7 days later. Mice fed 750 μg produced stronger T-cell responses to OVA than mice fed either 500 or 1000 μg (Fig. 3a) although all responses were substantial. These i.g. primed T cells when stimulated *in vitro* with antigen could produce IL-3/GM-CSF (Fig. 3a) but no IL-2 or IL-4 (Fig. 3b, c). The IL-3/GM-CSF response of MLN T cells obtained after i.g. priming was similar to that of lymph node cells from mice which had been primed subcutaneously with 100 μg OVA in CFA (Fig. 3a). It was found that the supernatants of i.g. primed T cells could only weakly stimulate the 32D cell line (in the experiments shown in Fig. 3 the maximum response induced by the IL-3 standard was 123,810 d.p.m., the response of the systemically primed cells was 80,510 d.p.m. and the i.g. primed MLN cells 14,440 d.p.m.). Therefore, this again indicates that most of the response measured by the FDC-P1 cell line is likely to be due to the release of GM-CSF. The proliferative response of MLN cells was also measured and consistent with the poor IL-2 response, i.g. primed T cells from the MLN proliferated only poorly *in vitro* in response to antigen (Fig. 3d).

To determine if T cells in other lymphoid organs share the same pattern of lymphokine secretion as the MLN cells following i.g. sensitization. BALB/c mice were treated with CY and fed 750 μg OVA and 1 week later the cells from the PP, MLN and spleen were cultured *in vitro* with antigen. In support of the previous experiments cells from each of the lymphoid tissues secreted GM-CSF and IFN-γ but did not produce IL-2 (Table 2).

To examine if the failure to produce IL-2 was due to a direct effect of CY, one group of BALB/c mice were treated with 200 mg/kg CY and 2 days later immunized subcutaneously with 100 μg OVA in CFA, while another group only received OVA in CFA. The periaortic and inguinal lymph nodes were assayed for T-cell responses to OVA 7 days later and as seen in Fig. 4 both groups of mice could produce IL-3/GM-CSF, IL-2 and IFN-γ respectively. Most noticeable was that T cells from CY-treated mice consistently produced higher levels of each of the lymphokines measured.

DISCUSSION

Feeding antigens to adult mice results in the induction of oral tolerance.[1] When mice were fed three times with 3 mg OVA they did not respond to systemic priming as measured by the ability of their lymph node cells to produce IL-3/GM-CSF. Yet despite being unresponsive by these criteria these mice were still highly sensitized when cells from the spleen and PP were examined for their ability to produce IL-3/GM-CSF *in vitro*. Thus PP and spleen cells produced large lymphokine responses when cultured with OVA. In contrast, MLN cells were unresponsive unless they were purified and cultured with spleen adherent cells. This phenomenon may be closely linked to the development of oral tolerance and thus requires further investigation. It may, however, simply be related to the superior antigen-presenting cell (APC) function of the spleen adherent cells since they could also stimulate stronger responses in purified PP T cells. We know from our previous studies that the spleen adherent fraction contains a large number of dendritic cells (DC).[14] These cells are known to express constitutively very high levels of class II major histocompatibility complex (MHC) and co-stimulatory activity and may therefore be able to present processed antigen much more efficiently to T cells *in vitro* than can be achieved by

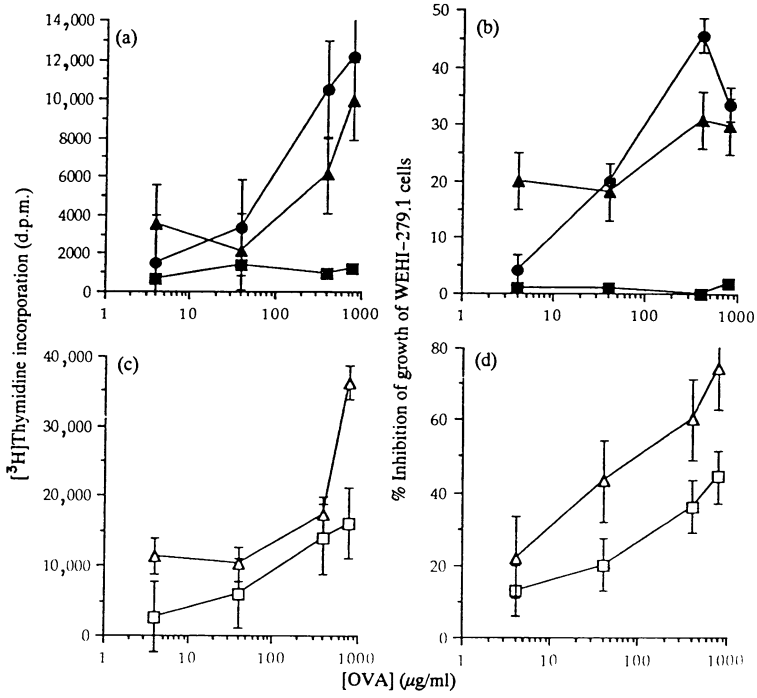

Figure 1. T-cell responses in the lymphoid organs of mice fed a tolerizing dose of OVA. (a, b) Mice were fed 3 mg of OVA over 3 days and after 1 week MLN (■), PP (▲) and spleen cells (●) were stimulated with antigen *in vitro*. Alternatively, (c, d), purified T cells from the MLN (□) and PP (△) were stimulated in the presence of splenic adherent cells. Supernatants were assayed for GM-CSF/IL-3 (a, c) and IFN-γ (b, d). The GM-CSF/IL-3 response measured by the FDC-P1 cell line was mainly due to GM-CSF since IL-3 release was minimal (i.e. in the presence of the IL-3 standard the 32D line gave a maximal response of 32,532 d.p.m., whereas with supernatants from tolerized mice it was 1398 d.p.m.). Background responses were < 500 d.p.m. Spleen adherent cells alone did not secrete IL-3/GM-CSF. Response of PP or MLN cells + spleen adherent cells versus PP or MLN cells alone is significantly different by Student's *t*-test ($P < 0.005$).

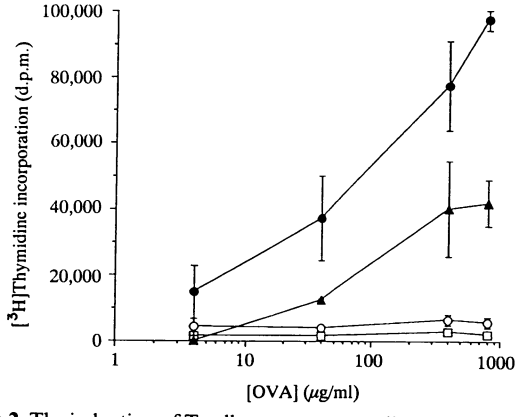

Figure 2. The induction of T-cell responses to orally administered OVA is dependent upon the dose of CY given. Mice were either untreated (□) or treated with 50 mg/kg (○), 100 mg/kg (▲) or 200 mg/kg body weight of CY (●) 2 days before being fed 750 μg OVA. Seven days later MLN cells were cultured *in vitro* and the level of IL-3/GM-CSF activity in the culture supernatants was assessed using the FDC-P1 cell line. Data represent the mean response of five individual mice ± SD.

the endogenous lymph node APC. At present it is difficult to discern whether the addition of spleen APC into lymph node cultures just increases the sensitivity of our assay system or whether spleen DC actually override the action of suppressor T cells. If the latter scenario were true, then the spleen DC may be able to provide a sufficient stimulus for the T cells which rescues them from tolerance. Since OVA-reactive T cells were not completely eliminated from lymphoid tissues after the first week following feeding, then it was not surprising that we observed a response in the PP and spleen. The PP is an important component of the gut-associated lymphoid tissues (GALT) and is thought to be the primary site where intestinally derived antigens are sampled by the immune system. The PP contains a large number of B cells that preferentially secrete IgA and are involved in providing protective immunity at the intestinal mucosa. We predict that the response observed in PP cells is mediated by OVA-specific IgA$^+$ B cells that would present antigen to T cells through receptor-mediated endocytosis. On the other hand, the response observed in spleen cell suspensions is likely to be stimulated by DC.

The lymphokines produced by T cells obtained from tolerized mice were very high levels of IFN-γ, moderate amounts of GM-CSF but no IL-2, IL-3 or IL-4. It is interesting to note that this pattern of lymphokine secretion is consistent with what has been reported for T-cell clones rendered anergic through exposure to high doses of synthetic peptides or by fixed APC.[15,16] O'Hehir *et al.* [15] have shown that anergic T cells display a distinct phenotype (i.e. high expression of cell surface CD4 and CD25, but low CD3) and we are currently in the process of examining whether lymphocytes obtained after oral tolerance might share a similar range of cell surface properties. The lack of IL-2 and IL-4 and the high IFN-γ responses of T cells may have a major influence on subsequent immunization. Both T- and B-cell responses require IL-2 and IL-4[17] and IFN-γ can inhibit the

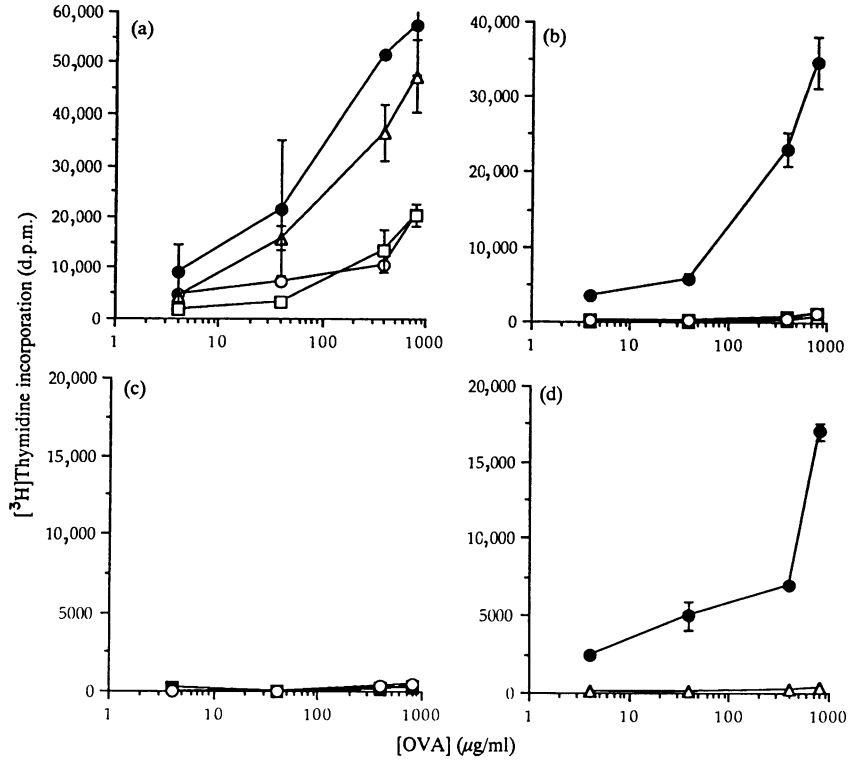

Figure 3. Intragastric priming of mice with OVA gives rise to a T-cell response which is equivalent to that induced following systemic immunization. Mice were immunized at the base of tail with 100 μg of OVA in CFA (●) or treated with 200 mg/kg CY and fed 500 μg (□), 750 μg (△) or 1000 μg OVA (○). The periaortic and inguinal lymph node cells from systemically primed mice and MLN cells from fed mice were assayed 7 days later for the response to OVA. (a) IL-3/GM-CSF; (b) IL-2; (c) IL-4. The proliferative responses of MLN cells from i.g. primed (△) and the periaortic and inguinal lymph node cells (●) from systemically primed mice was determined after 5 days in culture (d). The IL-3/CM-CSF response measured by the FDC-P1 cell line was mostly due to GM-CSF (see text). The peak responses of the IL-2 and IL-4 standards were 100,033 d.p.m. and 55,253 d.p.m. respectively and background responses for all assays were <900 d.p.m.

growth of Th2 cells[18] as well as the maturation of B cells,[13] and hence IFN-γ may have a strong negative influence on responses. It is possible that a deficit in antigen-specific B-cell maturation is a significant factor in the unresponsiveness of the MLN cultures. B cells have been shown to be an important class of APC in the regional lymph nodes of mice[19,20] and our own studies support this role for B cells, since we have found that lymph node DC are unable to process protein antigens but can present immunogenic peptides to primed T cells *in vitro*.[14] Dendritic cells which have been described in the mesenteric lymph[21,22] are, by analogy with other systems, important for priming naive T cells and recently it has been shown they can carry intestinally derived antigen in a processed form which can stimulate primed T cells *in vitro*.[22] Although DC may be involved in priming T-cell responses to orally administered antigens within the MLN, it is unlikely they will function in presenting exogenous antigens *in vitro*. Responses were normally measured in tolerized mice 1 week after feeding, and thus it may be simply that we did not allow enough time for the B cells to become activated within the MLN. It is not clear at this stage why the maturation of B cells within the MLN should be slower than that for the PP, but it could be influenced by the release of T-cell-derived lymphokines (e.g. IFN-γ). Michael has shown that intestinal processing of orally administered antigens can yield tolerogenic fragments of a protein and thus it may be important to consider what influence these fragments might have on the subsequent B-cell response.[23] One can at this stage only speculate as to what significance these factors might have on the mechanism that leads to the development of suppressor cells.

Under certain conditions feeding antigen can lead to systemic priming rather than tolerance. This can be achieved experimentally by treating mice with CY 2 days prior to feeding. The lymphokines produced by T cells stimulated by i.g. priming are documented here. The T-cell responses obtained after i.g. priming were equal in magnitude to those seen in the peripheral lymph nodes of mice immunized subcutaneously with OVA in CFA and produced high levels of GM-CSF, moderate to high levels of IFN-γ, low IL-3 and little or no IL-2 and IL-4. The low or absent levels of IL-2 were most notable so experiments were conducted to determine if this was an affect of CY *per se*. Treating mice with CY did not inhibit the ability of peripheral lymph node T cells to secrete IL-2 following subcutaneous immunization so the pattern of lymphokine secretion observed in i.g. primed T cells appears to be a result of the route of priming.

A major difference in the T-cell response observed after antigen feeding during tolerance induction versus i.g. priming is that the latter could stimulate a response in the MLN without the need for exogenous accessory cells. As mentioned above

Table 2. Comparison of lymphokine responses of cultures of the mesenteric lymph nodes, Peyer's patch and spleen cells from cyclophosphamide treated mice

	MLN	Peyer's patch	Spleen
*(a) IL-3/GM-CSF responses**			
[OVA] (μg/ml)			
800	38,152±6415	32,969±3828	26,565±13,204
400	14,145±1912	29,119±4376	17,112±8245
40	5906±1427	12,729±1242	12,104±1017
4	3366±290	8293±3228	1299±873
*(b) IL-2 responses**			
[OVA] (μg/ml)			
800	1063±329	750±320	2180±1025
400	756±47	800±190	1420±702
40	880±540	450±59	1662±823
4	882±250	350±120	777±324
(c) IFN-γ responses†			
[OVA] (μg/ml)			
800	20±3	38±8	45±15
400	6±2	42±6	17±2
40	5±1	14±4	4±2
4	1±1	1±1	2±1

* Results are the mean response (d.p.m. ±SD) of three mice. These experiments have been performed at least twice.

† Results are the % inhibition of growth (±SD) of the IFN-γ-sensitive cell line WEHI-279.1. Data are the mean response of three mice. Standards for the IL-2 assay gave 30,000 c.p.m.

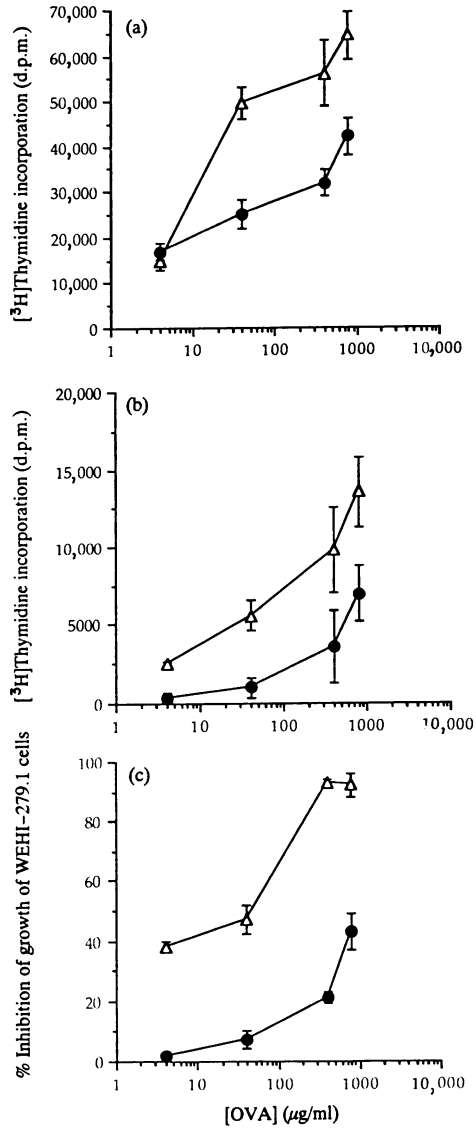

Figure 4. Treatment of mice with CY enhances the ability of mice to secrete lymphokines in response to subcutaneous antigen. Mice were treated with 200 mg/kg CY (△) or untreated (●) and immunized with 100 μg OVA in CFA. Seven days later the periaortic and inguinal lymph nodes were assessed for the release of: (a) IL-3/GM-CSF; (b) IL-2; (c) IFN-γ. Data are the mean response of triplicate cultures ±SD and this experiment has been repeated twice. The response of CY-treated mice versus untreated mice was significantly enhanced for each of the lymphokines measured (for IL-3/GM-CSF and IL-2 measurements, $P < 0.05$ and for IFN-γ, $P < 0.005$).

although DC are required for stimulating a primary antigen response in naive T cells, the B cell is an important APC required to stimulate the recall response to protein antigen *in vitro* by lymph node T cells. CY is toxic to B cells initially[24] but the capacity for renewal of these cells within the lymph node over the first week needs to be determined. CY also can act on DC to increase their accessory cell functions *in vitro*[24] so it could be envisaged then that during i.g. priming CY-treated DC may provide a stronger activation signal to the T cells bearing a specific receptor for epitopes presented on an orally administered antigen.

A question that remains to be resolved is how do T cells which respond to orally administered antigen undergo clonal expansion if they do not secrete IL-2 or IL-4. The simplest interpretation would be that these cells may only have a limited capacity to secrete T-cell growth factors. Thus when activated *in vivo*, i.g. primed T cells may only secrete IL-2 early after antigen exposure (i.e. within the first 24–48 hr). This short pulse of lymphokine production would enable the cells at least to undergo a limited amount of cellular proliferation but would restrict any overt reactions being elicited to the fed antigen. If this were the case then, the majority of the IL-2 produced by i.g. primed T cells *in vitro* may be rapidly consumed for their own growth, and thus this lymphokine may never accumulate to any excess in culture as would GM-CSF or IFN-γ.

In conclusion, these studies have shown for the first time that T cells sensitized to orally administered antigens display a limited capacity for lymphokine secretion *in vitro*. Since it is possible to measure T-cell responses during priming and tolerance we can now begin to study in more detail the presentation of antigens at mucosal surfaces. This information might therefore be useful in helping to elucidate the molecular mechanisms involved in producing oral tolerance.

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