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*J Immunol* 1999; 163:4728-4736; ;
http://www.jimmunol.org/content/163/9/4728

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Normal Induction of Oral Tolerance in the Absence of a Functional IL-12-Dependent IFN-γ Signaling Pathway

Allan Mcl. Mowat, Margaret Steel, Andrew J. Leishman, and Paul Garside

There is considerable evidence that regulatory cytokines play an important role in mediating the systemic tolerance found after oral administration of protein Ags. Although most existing work has focused on cytokines such as IL-4, IL-10, and TGF-β, recent evidence from TCR transgenic systems suggests that the induction of oral tolerance is accompanied by priming of Ag-specific IFN-γ production. IFN-γ has also been implicated as a mediator of T cell tolerance in other models in vivo and in vitro, including that induced by aerosol administration of protein. We show here that feeding tolerogenic doses of OVA primes for IFN-γ production in the spleen of mice with a normal T cell repertoire. However, depleting IFN-γ at the time of feeding OVA had no effect on the induction of tolerance. In addition, tolerance was induced normally in both IFN-γ receptor knockout (IFN-γR−/−) and IL-12 p40 knockout (IL-12−/−) mice. This was the case for all components of the systemic immune response and also with a variety of feeding protocols, including those believed to induce distinct regulatory mechanisms. We conclude that IL-12-dependent IFN-γ-mediated regulation does not play an essential role in oral tolerance. The Journal of Immunology, 1999, 163: 4728–4736.

The propensity of orally administered proteins to induce immunological tolerance is probably the physiological mechanism that prevents harmful reactions to food Ags (1). A similar mechanism may exist to control hypersensitivity to commensal bacteria (2–4). Interest in oral tolerance has also been stimulated in recent years by the demonstration that the oral route can be exploited as a means of delivering immunotherapy against autoimmune and inflammatory diseases (5–7). In addition, it is an important obstacle to the development of oral vaccines containing purified protein Ags. For these reasons, it would be important to establish the mechanisms involved, so that regimens for enhancing or preventing tolerance might be developed.

A variety of regulatory mechanisms have been implicated in oral tolerance, including anergy and deletion of Ag-specific T lymphocytes, as well as active suppressor mechanisms mediated by individual subsets of T cells or cytokines (5, 7). Of these, cytokine-dependent regulatory mechanisms would appear to be the most suitable targets for modulation. A number of different cytokines have been studied in oral tolerance, with most work focusing on the production of Th2 cell-dependent mediators such as IL-4 and IL-10, or on IL-10/TGF-β-producing Th3 or Tr1 CD4+ T cells (2, 7–13). However, IFN-γ produced by γδ T cells has been implicated as a mediator of the apparently analogous model of tolerance induced by aerosol administration of Ag (14, 15). This would be consistent with several other models of tolerance in vivo and in vitro, where the presence of T cell anergy is accompanied by preservation of IFN-γ production and, in some cases, can be prevented by neutralization of the cytokine (16–21).

Recent results suggest that the induction of oral tolerance may also be associated with early priming of IFN-γ production. Feeding tolerogenic doses of OVA to TCR transgenic mice, or recipients of TCR transgenic cells, stimulates IFN-γ production in the gut-associated lymphoid tissues (2, 11, 22). One interpretation of this is that the early release of IFN-γ is required for subsequent manifestations of tolerance, such as T cell anergy or active suppression. Here we have investigated whether IFN-γ-mediated events are essential for oral tolerance in mice by examining the effects of neutralizing IFN-γ in vivo and by inducing tolerance in mice lacking the IFN-γ receptor or the IFN-γ inducing cytokine, IL-12. Our results show that these pathways are not of central importance in the induction or expression of oral tolerance, irrespective of the immunoregulatory mechanism involved.

Materials and Methods

Animals

IFN-γR−/− 129/Sv and BALB/c IL-12 p40−/− mice were obtained originally from Dr. H. Bluethmann, Hoffmann La Roche, Basel, Switzerland, and Dr. J. Magram, Hoffmann La Roche, Nutley, NJ, respectively, before being bred and maintained in the animal facility at the University of Glasgow under specific pathogen-free conditions. In experiments with IL-12−/− mice, wild-type (WT)3 BALB/c mice bred in house were used, whereas in the studies of IFN-γR−/− animals, the WT mice were 129/Sv obtained from Harlan Olac, Bicester, Oxon, U.K. All mice were first used at 8–12 wk of age.

Induction and assessment of oral tolerance

Mice were fed OVA (Fraction V, Sigma, Poole, U.K.) dissolved in saline via a stainless steel gavage needle, and 10 days after the last feeding they were immunized with 100 μg OVA in CFA (Sigma) into one footpad. Control mice received 0.2 ml saline orally. Systemic immune responses were assessed in vitro by measuring OVA-specific proliferative activity and cytokine production in draining popliteal lymph nodes (PLN) 2 wk after immunization and in vivo by measuring systemic delayed-type hypersensitivity (DTH) and serum Ab responses 21 days after immunization.

Induction of priming by feeding OVA

Mice were fed 25 mg OVA, and 1, 2, 3, 4, and 10 days later, spleens, mesenteric lymph nodes, and Peyer’s patches were removed for culture in
Neutralization of IFN-γ in vivo

To deplete mice of IFN-γ during the induction of oral tolerance, mice were injected twice with 0.5 mg hamster IgG anti-mouse IFN-γ (R46A2, a gift of Celltech, Slough, U.K.) 1 day and 1 day before and 2 days after feeding 25 mg OVA. Control mice received 0.2 ml normal hamster serum, diluted 1:4 to obtain a concentration of 0.5 mg IgG.

Measurement of systemic immunity in vivo

As described previously (23), DTH responses were assessed by measuring the specific increment in footpad thickness 24 h after s.c. challenge of immunized mice with 100 μg heat-aggregated OVA in 50 μl saline using skinfold calipers, whereas the levels of OVA-specific total IgG, IgG1, and IgG2a isotypes in serum were measured by ELISA. In all assays, serial dilutions of a standard hyperimmune serum were included for calculation of the activity of test samples. The levels of total IgG are expressed as the percent activity of the hyperimmunized serum or as micrograms per ml IgG of the activity of test samples. The levels of total IgG are expressed as the titer obtained from determining the dilution of test serum which gave an OD equivalent to 5% of that found with the standard serum (23).

Measurement of OVA-specific proliferation and cytokine production in vitro

Single-cell suspensions were prepared in RPMI 1640 (Life Technologies, Gaithersburg, MD) by rubbing through a stainless steel mesh and passing the resulting suspension through Nitex mesh (Cadsich, London, U.K.). After three washes, the cells were resuspended at 10^6 cells/ml and cultured in 200-μl aliquots in flat-bottom 96-well tissue culture plates (Costar, Nucleopore, High Wycombe, U.K.) in RPMI 1640 containing 10% FCS, 100 U/ml penicillin, 100 mg/ml streptomycin, 2 mM l-glutamine, 25 mM HEPES, 50 mM 2-ME (all Life Technologies), either alone or with 1 mg/ml OVA. Proliferation was assessed by addition of 1 μCi/well [3H]Tdr for the last 18 h of culture. Cell-bound DNA was measured on filter mats, and [3H]Tdr incorporation was measured on a Betaplate counter. To measure cytokine production, 4 × 10^6 lymph node cells in 1-ml aliquots were cultured in 24-well tissue culture plates (Costar) either in medium alone or with 1 mg/ml OVA. Supernatants were harvested after 2–4 days and stored at −20°C until assayed. Cytokine production was quantified by sandwich ELISA techniques described in detail elsewhere (23), using appropriate pairs of capture and biotinylated detecting Abs (all Pharmingen, San Diego, CA). Ab binding was detected with extravidin-peroxidase (Sigma) and tetramethylbenzidine as described above. Cytokine concentrations in test supernatants were determined with reference to a standard curve constructed using serial dilutions of recombinant cytokines.

Statistical analysis

Results are represented as the mean ± SEM where indicated and were analyzed by Student’s t test, except for Ab levels, which were compared by the Wilcoxon rank sum test.

Results

Feeding tolerogenic doses of OVA primes Ag-specific IFN-γ production in vivo

Recent studies with TCR transgenic animals have suggested that feeding tolerogenic doses of Ag primes for the production of IFN-γ in the intestine-associated lymphoid tissues (2, 11, 22). To determine whether IFN-γ production is also primed during the induction of oral tolerance in normal animals, we fed BALB/c mice 25 mg OVA and assessed the ability of lymphoid cells to respond to restimulation with OVA in vitro.

Spleen cells from mice fed OVA 1, 2, or 3 days previously produced elevated levels of IFN-γ compared with saline-fed controls (Fig. 1). OVA-specific IFN-γ production was absent at later times. Spleen cells taken from mice fed OVA 1 or 2 days before also showed low levels of OVA-specific proliferative activity and IL-3 production when restimulated in vitro (data not shown), confirming the presence of T cell priming in normal mice fed a tolerogenic dose of OVA. A similar pattern of IFN-γ priming was also seen in the mesenteric lymph node of OVA-fed mice, but not in the mesenteric lymph nodes of saline-fed mice.

Oral tolerance is not influenced by neutralizing IFN-γ in vivo

In the first experiments, we determined whether oral tolerance could be induced in the absence of IFN-γ by administering a neutralizing anti-IFN-γ mAb around the time of feeding a tolerogenic dose of OVA. As expected, BALB/c mice treated with an isotype control Ab and fed OVA had significantly reduced systemic DTH and serum IgG Ab responses compared with saline-fed controls (Fig. 2, a and b). In parallel, these animals had significant tolerance of IgG2a Ab production (Fig. 2d). Saline-fed mice given anti-IFN-γ Ab had markedly reduced systemic immune responses compared with isotype-treated controls. However, systemic DTH (Fig. 2a) and serum IgG Ab (Fig. 2b) responses were tolerated significantly in mice fed 25 mg OVA and treated with anti-IFN-γ. Furthermore, the lower levels of IgG1 and IgG2a Ab production were also both significantly suppressed in OVA-fed, IFN-γ-depleted mice (Fig. 2, c and d).

Together, these findings do not support a role for IFN-γ in the induction of oral tolerance by feeding a high dose of Ag. However, because it remained possible that neutralization of IFN-γ by the...
Ab in these experiments was incomplete or that IFN-γ is critical for later phases of oral tolerance, we investigated the induction of tolerance in IFN-γR2/− mice. In addition, we attempted to exclude the possibility that the use of high dose Ag might obscure a subtle defect in oral tolerance by feeding a lower dose of 2 mg OVA as well as 25 mg OVA.

**Induction of oral tolerance in IFN-γR2/− mice**

Wild-type 129/Sv mice fed OVA showed dose-dependent suppression of subsequent DTH and proliferative responses after feeding OVA (Fig. 3, a and c). Ag-specific production of IFN-γ, IL-3, IL-5, and IL-10 showed similar dose-dependent tolerance in OVA-fed mice (Fig. 4). In addition, WT mice fed both doses of OVA had significantly reduced IgG1 and IgG2a Ab responses compared with saline-fed controls (Table I), although only the mice fed 25 mg OVA had tolerance of total IgG production (Fig. 3b).

Saline fed IFN-γR−/− animals had markedly reduced DTH responses compared with WT controls, and these responses were reduced almost to background levels by feeding either low or high dose OVA (Fig. 3a). OVA-specific total IgG responses were comparable in WT and IFN-γR−/− control mice after immunization (Fig. 3b), whereas OVA-specific IgG1 and IgG2a responses were significantly lower in saline-fed IFN-γR−/− mice (Table I). Feeding 2 or 25 mg OVA significantly inhibited all these humoral responses in IFN-γR−/− mice. OVA-specific proliferative responses of saline-fed IFN-γR−/− mice were significantly higher than those of WT controls, perhaps reflecting a normally cytostatic role for IFN-γ in this phenomenon (Fig. 3c). However, feeding either 2 or 25 mg OVA before immunization resulted in marked suppression of the proliferative responses of IFN-γR−/− cells, to levels equivalent to those seen in tolerant WT mice (Fig. 3c). OVA-specific production of IFN-γ was virtually negligible in cultures of saline-fed IFN-γR−/− cells, whereas IL-5 was produced in levels comparable with that of WT controls and OVA-specific IL-3 and IL-10 were significantly enhanced (Fig. 4). Despite these differences in the baseline cytokine levels in IFN-γR−/− and WT mice, feeding OVA to IFN-γR−/− mice before immunization markedly suppressed the production of all the cytokines. If anything, the tolerance of these cytokines was greater in IFN-γR−/− mice than in WT controls.

**FIGURE 2.** Effects of neutralizing IFN-γ in vivo on the induction of oral tolerance. OVA-specific immune responses 21 days after immunization with OVA/CFA. Mice were fed saline (Ctrl) or 25 mg OVA (Tol) 10 days before immunization and treated with 0.5 mg of either hamster IgG anti-IFN-γ Ab or normal hamster serum (NHS) 1 day before and 1 h before feeding. a, DTH responses expressed as the mean specific increment in footpad thickness 24 h after challenge with OVA. b, Total OVA-specific serum IgG responses expressed as percent hyperimmunized control serum. OVA-specific IgG1 (c) and IgG2a serum Ab (d) responses are shown as the reciprocal dilutions giving an OD value equivalent to IL-5 of hyperimmunized control serum. Results are the means ± SEM for six mice per group (*, p < 0.05 vs Ctrl given isotype control Ab; **, p < 0.05 vs saline fed Ctrl given anti-IFN-γ). STD, standard serum.
Taken together, these results show that oral tolerance of in vivo and in vitro responses is induced normally in the absence of endogenous IFN-\(\gamma\) function, with no defect in either its scope or its susceptibility.

**Normal induction of oral tolerance in IL-12\(^{-/-}\) mice**

To confirm that the induction of tolerance did not require the function of IFN-\(\gamma\) in vivo and to examine the proposed regulatory role for the IFN-\(\gamma\)-inducing cytokine IL-12, we examined the effects of feeding OVA to IL-12\(^{-/-}\) mice.

In the first experiment, we examined the tolerance induced by a single high dose feed of 25 mg OVA. Compared with wild-type BALB/c mice, saline fed IL-12\(^{-/-}\) mice had normal DTH, serum IgG (Fig. 5, a and b), IgG1, and IgG2a Ab responses (Table II) in vivo when challenged with OVA/CFA 10 days later, as well as normal OVA-specific proliferative responses (data not shown). However, saline-fed IL-12\(^{-/-}\) mice had somewhat higher OVA-specific IL-5 production than control BALB/c mice and, as anticipated, produced no IFN-\(\gamma\) (Fig. 5, c and d). With the exception of the defective IFN-\(\gamma\) response in IL-12\(^{-/-}\) animals, both groups of mice showed identical patterns of tolerance, with significant suppression of systemic OVA-specific DTH, total IgG, IL-5 (Fig. 5), IgG1, IgG2a (Table II), and proliferative responses (data not shown) in mice fed 25 mg OVA before immunization.

To explore further the susceptibility of WT and IL-12\(^{-/-}\) mice to the induction of tolerance, we next fed a lower dose of 2 mg OVA. In addition, we investigated whether the absence of IL-12 led to a selective defect in tolerance to multiple low doses of fed OVA, as might be expected from the evidence that this form of oral tolerance is mediated by TGF-\(\beta\)-mediated regulatory T cells which is counteracted by IL-12 (2, 11, 24). However, IL-12\(^{-/-}\) mice fed either 2 mg OVA on one occasion or 1 mg OVA on five occasions developed entirely normal tolerance of OVA-specific DTH (Fig. 6a), total IgG Ab responses (Fig. 6b), and proliferation (Fig. 6c). IgG1 Ab responses were also significantly suppressed in both WT and IL-12\(^{-/-}\) mice fed OVA in either regimen, as were IgG2a responses in the IL-12\(^{-/-}\) mice (Table III). WT mice had markedly reduced IgG2a production after feeding either 1 \(\times\) 2 mg or 5 \(\times\) 1 mg OVA, although this attained statistical significance only in mice fed multiple low doses of Ag (Table III). WT mice
fed OVA by either regimen had significantly suppressed OVA-specific IFN-γ production, but as before, no group of IL-12−/− mice had any OVA-specific IFN-γ production in vitro (data not shown). Once again, saline-fed IL-12−/− mice had enhanced OVA-specific IL-5 production compared with WT controls, but these exaggerated responses were dramatically reduced by feeding single or multiple low doses of OVA, similar to the effects in WT animals (Fig. 6d).

Thus, the absence of IL-12 does not alter the susceptibility of mice to oral tolerance, irrespective of the likely immunoregulatory mechanism.

Discussion

The results of the three separate experimental approaches presented here show that the induction of oral tolerance does not require the presence of functional IFN-γ. This conclusion is not dependent on the feeding regimen used and is despite the fact that IFN-γ production is primed during the induction phase of tolerance. Our findings contrast with evidence that IFN-γ is important in other models of peripheral tolerance and support the view that an IFN-γ-dependent regulatory mechanism does not play an essential role in oral tolerance to protein Ags.

Conflicting evidence has been presented recently on the role of IFN-γ in oral tolerance, with one study in IFN-γ−− mice reporting normal induction of tolerance after feeding multiple low doses of keyhole limpet hemocyanin (KLH) (25), whereas another found defective tolerance in IFN-γ−− mice fed a single high dose of OVA (26). Here we have reexamined these discrepancies using a combination of experimental approaches and by assessing a much wider range of systemic immune responses and more feeding regimens than used in previous studies. We show first that neutralizing IFN-γ at the time of feeding had no effect on the tolerance induced by feeding a single high dose of OVA. This regimen of Ab treatment was chosen because we found IFN-γ production to be primed very soon after feeding this dose and we had previously used the same Ab in a similar protocol to inhibit IFN-γ-producing Th1 cells (28–30). In both cases, feeding OVA resulted in an entirely normal state of tolerance identical in scope and intensity with that observed in WT animals. Importantly, the pattern of susceptibility of Th1- and Th2-dependent immune responses was entirely normal in both kinds of knockout (KO) mice fed single high or low doses of Ag, or using a multiple low dose feeding regimen, protocols that are believed to induce distinct regulatory mechanisms, clonal anergy/deletion, and active regulation, respectively (6, 7, 31, 32). Together, these results support the view that IFN-γ is not essential for any of these tolerogenic processes.
Our finding that IFN-γ plays no role in the induction or expression of oral tolerance contrast with other models of T cell tolerance in vivo and in vitro in which initial priming of IFN-γ production precedes an unresponsive state that requires the presence of the cytokine (17, 19, 20). In addition, it has been suggested that the tolerance induced by aerosol administration of OVA is dependent on IFN-γ-producing T cells (14, 15). Although this could reflect the involvement of distinct regulatory mechanisms in tolerance induced by different routes, others have failed to find an essential role for IFN-γ in peripheral tolerance (33). The mechanisms underlying such immunoregulatory effects of IFN-γ have also not been elucidated. It is more difficult to reconcile our findings with those of Kweon et al. (26) who reported a defect in oral tolerance in IFN-γ−/− mice fed high dose OVA. The defects in tolerance reported by these authors were generally small, with a significant loss of tolerance observed mainly in systemic B cell responses, whereas T cell-dependent responses such as DTH, proliferation, and cytokine production were tolerized effectively in the KO mice (26). Furthermore, others have supported our findings by reporting normal oral tolerance of Ab and cytokine production induced by feeding multiple low doses of KLH to IFN-γR−/− mice (25). It is possible that the apparent discrepancy between the susceptibilities of IFN-γ−/− and IFN-γ−/− mice to the induction of oral tolerance could reflect differences between these animals in immune function. Nevertheless, the fact that IFN-γ and its receptor form a unique receptor-ligand pair makes this idea seem unlikely.

An intriguing finding of our work was the IFN-γ production which was primed in the spleen soon after feeding a tolerogenic

Table II. OVA-specific serum IgG isotype responses in high dose orally tolerated IL-12−/− mice

<table>
<thead>
<tr>
<th>Fed</th>
<th>IgG1 anti-OVA</th>
<th>IgG2a anti-OVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>168 ± 33</td>
<td>139 ± 55</td>
</tr>
<tr>
<td>25 mg OVA</td>
<td>51 ± 10*</td>
<td>12.5 ± 1*</td>
</tr>
<tr>
<td>KO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>693 ± 199</td>
<td>306 ± 214</td>
</tr>
<tr>
<td>25 mg OVA</td>
<td>68 ± 0**</td>
<td>11.5 ± 3**</td>
</tr>
</tbody>
</table>

* OVA-specific IgG1 and IgG2a responses in BALB/c wild-type (WT) or IL-12−/− (KO) mice 21 days after immunization with OVA/CFA. Results are reciprocal dilutions giving an OD value equivalent to 5% of hyperimmunized control serum and are the means ± SEM for individual sera from five mice per group in animals fed saline, 1 × 2 mg OVA, or 5 × 1 mg OVA 10 days before immunization. **p < 0.05 vs WT control; ***, p < 0.05 vs IL-12−/− control. Similar results were obtained in a replicate experiment.
dose of Ag. This has now been described in the local and peripheral lymphoid tissues of Ag-fed animals in a number of normal and TCR transgenic models (2, 11, 22, 26, 34), and it is consistent with the idea that early IFN-γ production is part of the process of partial T cell activation that occurs in many forms of T cell tolerance and that may precede the onset of T cell anergy (16–21). Several pieces of evidence support the view that a similar process occurs in oral tolerance, including the fact that there is rapid but transient activation of Ag-specific T cells throughout the immune system of animals fed tolerogenic doses of Ag (Fig. 1 and Refs. 2, 11, and 34–37). In addition, the induction of oral tolerance is favored by presentation of Ag in the absence of adequate costimulation (1, 38), conditions that frequently lead to partial T cell activation and clonal anergy (16, 39). However, our current results indicate that the priming of IFN-γ production may be an epiphenomenon of partial T cell activation, rather than tolerance induction, and suggest that the two processes may not be linked mechanistically.

Recent studies have shown that exogenous IL-12 can prevent the induction of tolerance and act as a mucosal adjuvant (40) and can also partially reverse T cell anergy in vivo after parenteral administration of soluble peptide (41). However, we show here...
that tolerance to single high doses or multiple low doses of Ag was normal in IL-12−/− mice, confirming and extending our preliminary findings of normal oral tolerance to high dose KLH in IL-12−/− mice (42). Although a study published while our manuscript was in preparation indicates that all aspects of oral tolerance to a single high dose of OVA are normal in Stat4−/− mice that lack IL-12 signaling (43), ours is the first detailed examination of peripheral tolerance in the absence of IL-12. We conclude that IL-12 has no immunoregulatory role in oral tolerance to protein Ags in normal animals, irrespective of the mechanism involved, or dose of Ag fed. In addition, the normal tolerance in the absence of IL-12 demonstrates that oral tolerance of Th2 responses is not dependent on cross-regulation by Th1 cells, which do not develop in IL-12−/− animals. This contrasts with results reported in certain models in which Th2 responses can be suppressed by nasal administration of Ag (14, 15) but is analogous to the situation in which oral tolerance of Th1 responses does not require classical Th2 cells, as shown by studies in IL-4−/− mice (23, 44). Also, our study indicates that IL-12 is also not required for the uptake and processing events that underlie the ability of protein Ags to induce tolerance via the intestine.

It has been hypothesized that IFN-γ or IL-12 and TGF-β play mutually exclusive roles in determining the immune consequences of oral administration of Ag (2). According to this view, IL-12-dependent production of IFN-γ occurs during active immunity/inflammation, whereas TGF-β is associated with the induction of tolerance and is inhibited by IFN-γ (or IL-12). However, our own and other (26) studies show directly that tolerance to systemic challenge developed at the precise time when priming of IFN-γ production could be observed, indicating that the two processes are not necessarily exclusive. Further evidence against the idea that oral tolerance is dependent on an IL-12/IFN-γ TGF-β counterbalance is our observation that IL-12−/− mice did not show enhanced susceptibility to tolerance induction, even when a low dose feeding regimen was used, a protocol that is believed to selectively induce TGF-β-secreting regulatory cells (6). This is despite our earlier finding of increased TGF-β production in IL-12−/− mice (40). Others have also reported that the oral tolerance in Stat4−/− mice was not associated with an effect on TGF-β (43). Thus, the susceptibility of mice defective in IL-12 or IFN-γ does not necessarily correlate with differences in TGF-β production. This conclusion contrasts with the ability of anti-IL-12 Ab to cause parallel increases in both oral tolerance and TGF-β production in TCR transgenic mice fed OVA, as well as in mice fed soluble Ag to prevent hapten-induced intestinal immunopathology (2, 11, 24). Thus, the interactions among IL-12, IFN-γ, and TGF-β in the regulation of intestinal immune responses warrant further investigation in additional models.

In conclusion, our study indicates that IFN-γ and IL-12-dependent regulation of systemic immunity and other cytokines are not necessary for oral tolerance. However, priming of IFN-γ production appears to be a characteristic feature of the early mucosal immune response to Ag. Why this occurs and whether it is a unique property of the mucosal immune system remains to be clarified, as is the ultimate fate of the cells responsible. Elucidation of these processes will help our understanding of the induction and regulation of immune responses in these important tissues.

References


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