Human milk proteins including secretory IgA fail to elicit tolerance after feeding

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Abstract

Oral administration of large doses of protein antigen generally induces a state of systemic unresponsiveness currently termed mucosally induced tolerance. In this study, we used human milk protein (HMP) without casein as a multi-protein antigen for the study of mucosally induced tolerance. The HMP utilized in this study mainly contained secretory (S) IgA, lactoferrin (Lf) and α-lactalbumin (Lact). When mice were given 1 or 25 mg of HMP orally 3 times or 25 mg orally four consecutive weeks prior to systemic immunization, antigen-specific serum IgG responses to HMP were induced by subsequent parenteral immunization with 100 µg of HMP. Analysis of IgG subclasses revealed that IgG1 followed by IgG2b accounted for the IgG responses noted. When both HMP and ovalbumin (OVA) were fed to mice, tolerance developed to OVA but not to HMP. To further investigate the nature of immune responses seen following oral gavage of HMP, we examined responses to individual protein of HMP. Brisk serum IgG1 and IgG2b responses to both S-IgA and Lf were induced by oral followed by systemic immunization with HMP. Analysis of splenic CD4+ T cells from mice given oral HMP revealed production of Th2-type cytokines. These results show that oral administration of HMP preferentially induces exclusive Th2-type immune responses, which may prevent the development of HMP (S-IgA and Lf)-specific mucosally induced tolerance.

Introduction

Mucosally induced tolerance (including oral tolerance and nasal tolerance) is a state of antigen-specific systemic unresponsiveness induced by prior oral or nasal administration of proteins. More studies have been done by administering antigen by the oral route, and has been described in many species including mice, rats, guinea pigs, dogs, pigs and humans (1). The induction of tolerance is considered to be the basis of prevention of hypersensitivity reactions to dietary protein antigens (1). In fact, feeding bovine casein in the diet induced tolerance in mice (2,3). Recently, it has also been proposed as a strategy for treating a variety of autoimmune disorders (4,5). For example, this method of tolerance induction is presently being tested for the possibility of treating patients with multiple sclerosis, rheumatoid arthritis and autoimmune uveoretinitis (6).

The induction of mucosal tolerance has been demonstrated with a wide range of thymus-dependent protein antigens (1). In addition to soluble proteins, other tolerogenic antigens include heterologous red blood cells, inactivated bacteria and viruses (7–9). Several mechanisms have been put forth, and recent studies have shown that anergy, deletion and active suppression occurs in both effector CD4+ or CD8+ T cells and their derived cytokines [e.g. transforming growth factor (TGF)-β] (6). In addition, mucosally induced tolerance may be associated with dysregulation of Th1 and Th2 cells (10). However, the exact mechanisms for the induction of this unique systemic
unresponsiveness induced by oral antigen administration still remains to be elucidated.

The most extensively studied model of oral tolerance to a protein has been the oral administration of ovalbumin (OVA) to mice, where systemic immune responses can be suppressed after a single feeding of a large dose of OVA (11–12). Although oral administration of a large bolus of purified protein generally induces systemic unresponsiveness, few studies have used mixtures of proteins which would more naturally mimic the development of unresponsiveness to food or milk proteins. In this study, we used human milk protein without casein (HMP) as a multi-protein antigen to study oral tolerance. The HMP consists mainly of secretory (S) IgA, lactoferrin (Lf) and α-lactalbumin (Lact). During our studies of oral tolerance to these proteins, we noted that oral administration of a mixture of select HMP failed to induce systemic unresponsiveness. This study describes the nature of host immune response to HMP in this model.

Methods

Mice

C57BL/6 mice were obtained from the Frederick Cancer Research Facility (National Cancer Institute, Frederick, MD) at 6–8 weeks of age. Mice were maintained in horizontal laminar flow cabinets, and were provided sterile food and water ad libitum.

HMP

Samples of human milk, obtained from 10 healthy mothers 2–7 days after delivery, were pooled and kept frozen at −25°C until used. HMP was purified from human milk as follows. Fat was removed by centrifugation at 38,000 g for 30 min at 4°C. Casein was partly removed by adjusting the pH to 4.6, followed by precipitation for 1 h at 4°C and centrifugation at 38,000 g for 1 h at 4°C. HMP was collected by a salting out procedure using 50% ammonium sulfate at pH 5.0, and then dialyzed and lyophilized in the presence of lactose. The protein components of HMP mainly consisted of HMP S-IgA, Lf and Lact, and their compositions were 12.44% for HMP S-IgA, 39.52% for Lf and 31.49% for Lact respectively. Doses of HMP were expressed as mg or µg protein in this study. The individual proteins were purified by the standard method of others (13–15). OVA was purchased from Sigma (St Louis, MO).

Immunization

Mice were given HMP and/or OVA by gavage using a stainless steel gavage needle as routinely performed in our group (16). The desired amount (1 or 25 mg) of antigen was administered 3 times at weekly intervals in 0.25 ml of saline. In some experiments, mice were given 25 mg of HMP for 28 days. One week following the last oral dose, mice were parenterally immunized [e.g. i.v. (HMP only) or s.c. with complete Freund’s adjuvant (CFA; Difco, Detroit, MI), HMP and OVA experiments)] with the same antigen (100 µg). Blood samples were collected from different groups of mice at weekly intervals.

Detection of antigen-specific antibody by ELISA

HMP S-IgA, Lf, Lact or OVA specific antibody titers in serum were determined by ELISA as described previously (17). Briefly, Falcon Microtest III assay plates (Becton Dickinson, Oxnard, CA) were coated with a HMP S-IgA (100 µl of 5 µg/ml), Lf (100 µg of 10 µg/ml), Lact (100 µl of 50 µg/ml) or OVA (100 µl of 1 mg/ml) in PBS. Wells were blocked with 200 µl of PBS containing 1% BSA for 1 h at 37°C. After washing, serial 2-fold dilutions of serum were added and the plates were incubated for 2 h at 37°C. Similar dilutions of serum from non-immunized mice were included as controls. After incubation, the plates were washed and a secondary antibody consisting of 100 µl of a 1:1,000 dilution of horseradish peroxidase-conjugated goat anti-mouse µ, γ or α heavy chain specific antibody (Southern Biotechnology Associates, Birmingham, AL) was added to the plates. The plates were incubated at 37°C for 2 h and, after washing, developed at room temperature with 100 µl of the chromogenic substrate, 3,3',5,5'-tetramethylbenzidine with H2O2 (Moss, Pasadena, MD). Reactions were terminated by addition of 50 µl of 0.5 M HCl. For IgG subclass determination, biotinylated mAb specific for IgG1, IgG2a, IgG2b and IgG3 (Pharmingen, San Diego, CA) and streptavidin-conjugated peroxidase were employed. Endpoint titers were expressed as the reciprocal log2 of the last dilution which gave a reading of OD 450 nm of 0.1 units higher than negative controls after a 15 min incubation.

Antigen-specific ELISPOT

The method for isolating spleen cells was described in detail elsewhere (18). Cells were resuspended in complete medium (RPMI 1640; Cellgro Mediatech, Washington, DC) containing 10% FCS, HEPES buffer (15 nM), L-glutamine (2 mM), penicillin (100 U/ml) and streptomycin (100 µg/ml). HMP S-IgA-, Lf- and Lact-specific IgM, IgG and IgA antibody-forming cells (AFC) were enumerated in cell suspensions by antigen-specific ELISPOT assays (19). Briefly, 96-well nitrocellulose-based plates were coated with 5 µg/ml of HMP S-IgA, 10 µg/ml of Lf or 50 µg/ml of Lact diluted in PBS for the enumeration of antigen-specific AFC. Control wells received PBS only. Wells were blocked with 10% FCS/PBS. Serial 5-fold dilutions of splenic mononuclear cells starting at 1×10^6 cells were added to these wells in duplicate and incubated for 4 h. Spots were developed with peroxidase-labeled anti-mouse µ, γ or α (Southern Biotechnology Associates) and visualized by adding the chromogenic substrate, AEC (Moss). Spots were counted with the aid of a dissecting microscope (SZH Zoom Stereo Microscope System; Olympus, Lake Success, NY).

Antigen-specific T cell proliferation

For purification of CD4^+ T cells from the non-adherent spleen cell suspensions, a MACS system was used (Stemfen Miltenyi Biotechnological Equipment, Bergish-Gladach, Germany) as previously described (20). Spleen cells were first incubated with biotinylated anti-CD3 (OKT4) and streptavidin-conjugated microbeads. Cells adherent to magnetic beads were separated using a magnetically charged column as described elsewhere. Purified CD4^+ T cells were then re-stimulated in vitro with antigen according to previously described

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Results

Failure to induce oral tolerance to HMP

Groups of C57BL/6 mice were orally administered with saline or various doses of HMP followed by systemic immunization (i.v.) with 100 μg of HMP. One week after final immunization, IgG antibody titers to the three major antigens, HMP S-IgA, Lf and Lact specific, were determined by ELISA. Oral administration of HMP did not affect systemic HMP S-IgA- and Lf-specific IgG antibody responses induced by after immunization (Fig. 1A). On the other hand, Lact-specific IgG antibody responses were not induced (data not shown). These results show that oral administration of HMP did not induce oral tolerance to HMP S-IgA or Lf. Analysis of IgG subclass antibody responses to HMP S-IgA and Lf revealed that antigen-specific IgG1 followed by IgG2b accounted for all the HMP S-IgA- and Lf-specific IgG responses (Fig. 1B). In order to further confirm our findings, additional groups of mice were given HMP by the oral route and then systemically immunized via the s.c. route instead of i.v. Thus, mice were challenged with the same antigen in the presence of CFA. A similar pattern of HMP-specific immune responses was also noted (Fig. 1C). Two groups of mice immunized s.c. with HMP once (control 1) or twice (control 2) without any prior oral treatment with HMP resulted in the induction of S-IgA- and Lf-, but not Lact-specific IgG responses. Further, similar levels of S-IgA- and Lf-specific antibody responses were also maintained in the groups of mice that received oral HMP before s.c. immunization (Fig. 1C). Taken together, these results suggest that systemic unresponsiveness was not induced to HMP.

HMP fails to prevent oral tolerance to co-administered OVA

To investigate whether or not HMP or individual protein components of this molecule may possess the ability to prevent the development of oral tolerance to other protein antigens, groups of C57BL/6 mice were fed saline, 20 mg of OVA or 20 mg of OVA plus 25 mg of HMP 3 times per week for 2 weeks and all mice were immunized s.c. with 100 μg of OVA plus CFA twice at weekly intervals. One week after the final immunization, OVA-specific IgG antibody titers were determined by ELISA. Feeding OVA alone or OVA with HMP

Table 1. CD4⁺ T₃₁ and T₃₂ cytokine-specific mAbs and recombinant cytokines used for ELISA

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Coating mAbᵃ</th>
<th>Detection mAbᵃ</th>
<th>Recombinant cytokine concentration range</th>
<th>Origin</th>
</tr>
</thead>
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<tr>
<td>IFN-γ</td>
<td>R4-6A2</td>
<td>XMG1.2</td>
<td>800–0.8 (U/ml)</td>
<td>Genzyme</td>
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<tr>
<td>IL-2</td>
<td>JE56-1A12</td>
<td>JE56-9H4</td>
<td>200–0.2 (U/ml)</td>
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<tr>
<td>IL-4</td>
<td>BVD4-1D11</td>
<td>BVD6-24G2</td>
<td>25–0.025 (ng/ml)</td>
<td>Endogen</td>
</tr>
<tr>
<td>IL-5</td>
<td>TRFK-5</td>
<td>TRFK-3</td>
<td>200–0.2 (U/ml)</td>
<td>Genzyme</td>
</tr>
<tr>
<td>IL-6</td>
<td>MPS-30F3</td>
<td>MPS-32C11</td>
<td>25–0.025 (ng/ml)</td>
<td>Genzyme</td>
</tr>
<tr>
<td>IL-10</td>
<td>JE5-2A5</td>
<td>SXC-1</td>
<td>1250–1.25 (U/ml)</td>
<td>R&amp;D Systems</td>
</tr>
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ᵃAll antibodies were obtained from PharMingen.
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Fig. 1. Lack of systemic unresponsiveness following oral administration of HMP to mice. Mice were orally administered with saline (controls) or 1 mg (oral 1) or 25 mg (oral 2) of HMP 3 times at weekly intervals or 25 mg daily for 4 weeks (oral 3) followed by i.v. immunization with 100μg of HMP. One week after final immunization, antigen-specific IgG from all groups (A) and IgG subclasses from oral 3 (B) titers in serum were determined by ELISA. In the other experimental groups (C), mice were orally administered with saline (control 1), 1 mg (oral 1) or 25 mg (oral 2) of HMP 3 times at weekly intervals followed by s.c. immunization with 250μg of HMP in CFA. An additional group of mice was primed s.c. with HMP (250μg), and then immunized with HMP and CFA (control 2). Serum samples from these mice were also tested for antigen-specific IgG responses by ELISA. Results presented are the mean ± SD (nine mice per group) and were from three separate experiments.

resulted in lower levels of OVA-specific serum antibody responses when compared with control groups. This finding showed that oral tolerance to OVA was induced regardless of the presence or absence of HMP. When similarly treated mice were immunized systemically with HMP, S-IgA- and Lf-specific antibody responses were noted (data not shown). Thus, HMP does not harbor the ability to prevent the development of oral tolerance to co-administered, unrelated antigen.

Analysis of antigen-specific antibody responses in mice orally immunized with HMP

To investigate the possible mechanism(s) for the lack of oral tolerance induction by HMP, we next examined antigen-specific immune responses induced by oral administration with HMP. As controls, the other groups of mice were immunized systemically with HMP instead of by the oral route. One week after the last immunization, serum samples were obtained and analyzed for isotype and HMP-specific responses by ELISA. Brisk serum IgG responses for HMP components of S-IgA and Lf were induced by both oral and systemic immunization (Figs 3 and 4). Further, analysis of serum IgG subclass responses revealed that HMP S-IgA- and Lf-specific IgG antibodies were represented by mainly IgG1 followed by IgG2b subclasses. In contrast to these HMP S-IgA- and Lf-specific antibody responses, Lact-specific IgG antibodies were induced by neither oral nor systemic immunization with HMP (Figs 3 and 4).

To further confirm these findings, spleen cells were isolated from mice immunized orally or systemically with HMP. When HMP-specific AFC were examined by ELISPOT assay, HMP S-IgA- and Lf-specific IgG antibody producing cells were induced in spleen of mice immunized orally or systemically with HMP (Fig. 5). However, Lact-specific IgG antibody-producing cells were not detected (data not shown). These findings show that HMP, especially S-IgA and Lf, are strong immunogens for the induction of antigen-specific immune responses even when administered via the oral route without any mucosal adjuvant. Further, a profile of antigen-specific IgG subclass responses suggested that Th2-type immune responses were induced by oral administration with HMP.

Analysis of antigen-specific T cell responses in mice orally immunized with HMP

To investigate antigen-specific T cell responses induced by orally administered HMP, we characterized Th1/Th2 cytokine production by in vitro antigen-stimulated CD4+ T cells isolated from mice immunized orally or systemically with HMP. Maximal T cell proliferation was achieved when CD4+ T cells (2x10^6/ml) from spleens of orally immunized mice were incubated with 1x10^6 feeder cells/ml, 20 U/ml of IL-2 and 100 μg/ml of HMP S-IgA or Lf (Fig. 6). The levels of oral antigen-induced T cell responses were comparable to those
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Fig. 3. Analysis of antigen-specific immune responses in mice orally immunized with HMP. Mice were immunized orally with 1 mg of HMP 3 times at weekly intervals. One week after the last immunization, the major serum isotypes (A) and IgG subclasses (B) responses for anti-HMP antibodies were determined by ELISA. HMP-specific IgA was not tested for HMP S-IgA. The results were taken from three separate experiments (five mice per group) and were expressed as the mean ± SD.

Fig. 4. Analysis of antigen-specific immune responses in mice i.v. immunized with HMP. Mice were immunized i.v. with 100 µg of HMP twice at weekly intervals. One week after the last immunization, the major isotypes (A) and IgG subclasses (B) of serum HMP-specific antibodies were determined by ELISA. Antigen-specific IgA was not tested for HMP S-IgA. The results were taken from three separate experiments (five mice per group) and were expressed as the mean ± SD.

Fig. 5. Characterization of antigen-specific B cell responses in mice orally immunized with HMP. Mice were immunized orally with 1 mg of HMP 3 times or i.v. with 100 µg of HMP twice at weekly intervals. One week after the last immunization, antigen-specific AFC from spleens of mice orally (A) or i.v. (B) immunized with HMP were examined by ELISPOT assay. Antigen-specific IgA was not tested for HMP S-IgA. Results were from three separate experiments (five mice per group) and were expressed as the mean ± SD.
obtained by systemic immunization. This finding indicates that antigen-specific T cells are induced by oral administration of HMP and neither T cell anergy nor clonal deletion was induced by this immunization regimen.

The culture supernatants were examined for T1 and T2 cytokine synthesis by ELISA. CD4+ T cells from spleens of orally immunized mice produced T1-type cytokines, IL-4, IL-5, IL-6 and IL-10, but not T1-type cytokines, IFN-γ and IL-2 (Fig. 7). A similar pattern of antigen-specific T1 and T2 cell responses (e.g. T2 > T1) was also noted in the systemically immunized control group. Since rIL-2 was added in these T cell cultures, detectable levels of IL-2 were found in the culture supernatants; however, the level of rIL-2 was equivalent to that of control cultures (without antigen). These results suggest that oral administration of HMP induces exclusive T2-type immune responses which lead to the maintenance of antigen-specific immune responses in the systemic compartment.

Discussion

The present study has shown that a multi-protein human mucosal product, i.e. S-IgA/Lf, was unable to induce oral tolerance when given to mice. Thus, administration of HMP by standard immunization protocols used for the induction of oral tolerance to other proteins such as OVA, where large doses of protein antigen were given via the oral route followed by systemic challenge, did not result in the systemic unresponsiveness to HMP. Since the HMP preparation used in this study contained three main milk proteins including S-IgA, Lf and Lact, it was important to dissect antigen-specific immune responses induced to these three major proteins. This lack of oral tolerance was associated with serum IgG antibody responses to HMP S-IgA and Lf, but not to Lact (Fig. 1). Lact did not induce significant IgG antibody responses even when administered by a systemic route.

Although we do not have any specific explanation for the lack of Lact-specific immune responses [Figs 1A (control), 1C (controls 1 and 2) and 1F], this may be an attribute of the molecule itself. On the other hand, the other two components of HMP possess strong immunogenicity when HMP was given by either the oral or parenteral routes. Thus, one simple explanation for the lack of oral tolerance to HMP could be a high innate immunogenicity of HMP, and especially for S-IgA and Lf. Thus, it is interesting to postulate the possibility that formation of multiple components of proteins such as HMP could be a practical method to elicit immune responses.

In order to understand the immunological basis for lack of oral tolerance induced by HMP, mice were given HMP by the oral route, and HMP-specific responses were examined by the analysis of serum and splenic mononuclear cells from these mice with HMP using ELISA and ELISPOT assays, high levels of HMP-specific IgG antibody and increased numbers of IgG AFC were noted respectively (Figs 3–5). It is important to point out that HMP-specific IgG responses were induced by oral immunization without co-administration of mucosal adjuvants such as cholera toxin (CT). To this end, it has been shown that co-administration of mucosal adjuvant is essential for the induction of good antigen-specific antibody responses in systemic compartments by oral immunization with soluble protein antigens (17,22,23). Further, assessment of these antigen-specific IgG responses induced by oral immunization revealed that IgG subclasses of HMP S-IgA- and Lf-specific antibodies were exclusively IgG subclasses γ1 and γ2b.

An outcome of antigen-specific IgG subclass responses suggested that orally administered HMP induced T2-type responses. In this regard, in vitro antigen re-stimulation of CD4+ T cells from spleens of orally immunized mice with HMP resulted in the production of IL-4, IL-5, IL-6 and IL-10 but not IFN-γ and IL-2 (Fig. 7). This profile of cytokine production by antigen-specific splenic CD4+ T cells strongly suggested that HMP-specific T2-type responses were induced by oral administration with HMP. Thus, a preferential induction of T2-type responses by oral HMP may prevent the development of systemic unresponsiveness. Thus, production of T2 cytokines such as IL-4 by HMP-specific CD4+ T cells in the peripheral site allows induction of antigen-specific IgG1 and IgG2b antibody responses. To this end, IL-4 produced by T2 cells has been shown to support the induction of IgG1 responses (24).

Several possible mechanisms for the induction of oral tolerance have been proposed, which include: (i) induction of T cell anergy and clonal deletion (25), (ii) generation of active suppression by CD4+ or CD8+ T cells producing cytokines capable of suppressing immune responses (e.g. TGF-β) (26), and (iii) dysregulation between T1 and T2 cells (10). Considering the third possibility, it has been reported that T1 cells are more likely to be anergized than T2 cells (27). Therefore, oral tolerance has been shown to reflect preferential activation of T2 cells with subsequent down-regulation of T1 responses (28). However, recent studies by

![Image](image-url)
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<th>Route</th>
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<th>Th2 Cytokines</th>
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<td>Oral</td>
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Fig. 7. Analysis of Th1- and Th2-type cytokine production by HMP-specific splenic CD4+ T cells isolated from mice orally or i.v. immunized with HMP. Identical immunization protocols and T cell culture conditions were used in this experiment as described in Fig. 6. Culture supernatants were harvested following 4 days of incubation and then analyzed by the respective cytokine-specific ELISA. Results were from three separate experiments (six mice per group). *P <0.05 when compared with the non-stimulated group.

others demonstrated that both Th1- and Th2-type cells are equally susceptible to oral tolerance (10). Our present findings provided a different view that induction of CD4+ Th2-type responses in systemic compartments by oral feeding with a multi-component protein, HMP, resulted in the prevention of development of antigen-specific systemic unresponsiveness. In this regard, it has been shown that CT as mucosal adjuvant possesses an ability to abrogate oral tolerance (29). Further, our previous studies have shown that orally administered CT can induce strong Th2-type responses in both systemic and mucosal compartments which accounted for the generation of antigen-specific IgG and IgA responses respectively (23). Thus, in the situation of oral tolerance, oral administration of CT may alter the balance of Th1 and Th2 cells toward a dominant Th2-type response which allowed abrogation of systemic unresponsiveness. Our most recent and separate studies using IFN-γ−/− mice which express dominant Th2 cells with an absence of Th1-type responses demonstrated that oral tolerance was not developed following oral administration of a large dose of OVA (30). Thus, oral administration of 25 mg OVA in IFN-γ−/− mice resulted in the induction of sufficient levels of Th2 cytokines which allowed the maintenance of antigen-specific antibody and B cell responses in the systemic compartment. Taken together, the findings provided by the previous study (30) and by the present investigation suggested that induction of preferential Th2-type responses prevents the development of systemic unresponsiveness following oral administration of large amounts of protein antigen. It is also possible that these CD4+ Th2-type T cells only produced classical Th2 cytokines, e.g. IL-4, IL-5 IL-6 and IL-10 but not TGF-β, while in other investigations, TGF-β-producing T cells were found in conjunction with elevated synthesis of the Th2 cytokines IL-4 and IL-10 (31). Thus, our future studies will address this issue as to whether or not oral HMP-induced Th2 cells produce TGF-β in addition to an array of classical Th2-type cytokines.

Another intriguing finding of the study is that of the different constituents of HMP, both S-IgA and Lf, but not Lact possessed strong immunogenicity for the induction of antigen-specific IgG1 and IgG2b antibody responses following oral administration without a mucosal adjuvant. In general, orally encountered proteins are disintegrated into small peptides by digestive enzymes during enterokinesis and then absorbed via the epithelium. To this end, components of OVA have...
been detected in the serum of orally administered experimental animals (32). In the case of HMP S-IgA and Lf, these proteins may be taken up by different processes where specific receptors may be involved. In fact, intestinal epithelial cells have been shown to express both Fc and polymeric Ig receptors (33). It is also clear that Fc receptors are expressed on the apical portion of epithelial cells in addition to the polymeric Ig receptor on the basolateral portion of membranes (34,35). In addition, immunocompetent cells such as lymphocytes and macrophages have been shown to express Lf receptors (36,37). It was also suggested that murine lymphocytes and macrophages express an Fe receptor-like molecule which can bind to IgA (38,39). It is possible that binding of HMP S-IgA and Lf to these specific receptors expressed by epithelial cells and by lymphocytes/macrophages may enhance immunogenicity of these proteins. Alternatively, orally fed HMP S-IgA and Lf could be directly bound to epithelial cells via respective receptors which may lead to absorption of intact molecules without peptide fragmentation by digestion. Thus, whole protein molecules could be presented to the host immune system. As a result, strong antigen-specific immune responses could be induced by S-IgA and Lf but not Lact. Finally, signaling provided through these receptors upon engaging with S-IgA and Lf may inhibit the process of T cell anergy or apoptosis which may account for the maintenance of antigen-specific immune response in the setting of oral tolerance. These possibilities are currently being examined in our ongoing study.

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Abbreviations

- AFC: antigen-forming cells
- CFA: complete Freund's adjuvant
- CT: cholera toxin
- HMP: human milk protein
- Lact: α-lactalbumin
- Lf: lactoferrin
- OVA: ovalbumin
- S: secretory
- TGF: transforming growth factor

References

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