# The Role of Chemokines in Oral Tolerance

## Abrogation of Nonresponsiveness by Treatment with Antimonocyte Chemotactic Protein-1[a]

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## INTRODUCTION

Peripheral, antigen-specific tolerance can be induced by oral administration of both protein and hapten antigens.[1] Oral tolerance has been used to prevent the induction of experimental autoimmune diseases[2–6] and is currently being used in clinical trials for treatment of human autoimmune diseases.[7,8] The mechanism of oral tolerance is not completely understood. There have been reports suggesting that feeding antigen induces both anergy and regulatory T cells.[9–13] More recently, the mechanism of oral tolerance has been reported to be induction of anergy/deletion by feeding a high dose of antigen[14] or regulatory T cells that secrete TGF-β by feeding multiple, small doses of antigen.[15,16] Introduction of antigen into mucosal surfaces appears to inhibit peripheral cell-mediated immune responses[9,17] but primes mucosal antibody responses[18] and also peripheral antibody responses.[19,20] Events at the intestinal mucosa that modulate antigen uptake and processing (*e.g.*, inhibition of oral tolerance induction by introduction of cholera toxin and antigen) also appear to have an influence on whether feeding antigen induces peripheral tolerance or primes a peripheral immune response.[17,21–27] Additionally, the ability to generate an IL-4 response at the time of feeding also determines whether oral tolerance can be induced.[28] Whether introduction of antigen by feeding leads to peripheral tolerance or primes a peripheral immune response, it appears that migration of lymphoid cells in and out of the mucosal immune system to and from the peripheral immune system is a necessary component of oral tolerance.[11,29,30]

Lymphocytes leave the circulation, migrate, and accumulate at sites of inflammation in response to chemoattractants. Historically, leukotrienes, platelet-activating factor (PAF), and C5a have been defined as nonspecific chemoattractants responsible for mononuclear cell recruitment. Recently, several laboratories have identified specific chemoattractant cytokines (chemokines) for neutrophils as well as mononuclear and lymphoid cells. The

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potent factors are divided into two highly conserved gene families: C-x-C and C-C, designated by the position of the first two cysteines.[31,32] The C-x-C (or α) chemokines are primarily chemotactic for neutrophils.[33] The prototypic C-x-C chemokine is IL-8. The C-C (or β) chemokines are primarily chemotactic for mononuclear cells, basophils, and eosinophils.[33–35] Representatives of this family include macrophage inflammatory protein (MIP)-1α and monocyte chemotactic protein (MCP)-1. The cellular source of the C-C chemokines has only recently been elucidated. MIP-1α has been shown to be expressed primarily by mononuclear cells, neutrophils, and inflammatory fibroblasts[36] and also has been shown to up-regulate ICAM-1,[37–40] demonstrating the importance of this factor in the migration of mononuclear cells. Another member of the C-C family, MCP-1, was first identified as an early response gene (JE) in murine fibroblasts treated with PDGF.[41] MCP-1 expression has been demonstrated in monocytes, lymphocytes, endothelial cells, epithelial cells, and smooth muscle cells[36,42] and has been identified as a chemotactic factor for $CD4^+$ T cells.[43,44] Recently, infection of intestinal epithelia has been shown to induce the production of MCP-1.[45]

Inasmuch as migration of lymphocytes seems to be an important consequence or oral tolerance induction, we hypothesized that C-C chemokines might be important factors in the induction of oral tolerance. In the present report, we tested the *in vivo* role of MIP-1α and MCP-1 in the induction of oral tolerance to human gamma globulin (HGG).

## MATERIAL AND METHODS

### *Mice*

Inbred female SJL/J ($H\text{-}2^s$) mice were purchased from Harlan Sprague Dawley (Indianapolis, IN) and housed according to Northwestern University and NIH guidelines.

### *Antigens*

HGG was purchased from Sigma (St. Louis, MO). Bovine MBP was a kind gift of Dr. Stephen D. Miller (Northwestern University).

### In Vitro *T-cell Proliferation/Cytokine Assays*

*In vitro* proliferative responses of lymph node T cells were measured according to established methods using [$^3$H]TdR incorporation.[46] Single cell suspensions from pooled lymph nodes were cultured at a density of $2 \times 10^6$ cells/mL in Dulbecco's modified Eagle medium (DMEM) containing 5% FCS, 1 mM glutamine, 1% Pen-Strep, 1 mM nonessential amino acids, and $5 \times 10^{-5}$ M 2-ME (complete DMEM-5, all components from Sigma) in the presence or absence of 5 μg/mL HGG. Antigen-induced cytokine production was assayed from 24-, 48-, and 72-h culture supernatants. Duplicate samples were tested for the presence of IL-2, IL-4, and IL-10 by ELISA, using cyto*screen*™ ELISA kits (Biosource International, Camarillo, CA). Transforming growth factor (TGF)-β was analyzed from supernatants of cells grown in serum-free medium using a modified ELISA as previously described.[15] IFN-γ production was measured by capture ELISA using recombinant cytokine as a standard.[46] Measurement of MIP-1α and MCP-1 was performed on cell culture supernatants, as previously described.[36]

### *Induction of Antigen-specific DTH*

Prechallenge ear thickness in metofane-anesthetized animals was measured with a Mitotoyo dial thickness gauge. Five μg of antigen (in 10 μL PBS) was injected intradermally into the dorsal surface of the ear using a 100 μL Hamilton syringe fitted with a 30 g needle. Ear swelling was measured 24 h later and expressed in units of $10^{-4}$ inches. HGG-induced ear-swelling responses are the result of mononuclear cell infiltration and show typical delayed-type hypersensitivity (DTH) kinetics (*i.e.*, minimal swelling at 4 h, maximal swelling at 24-48 h).

### In Vivo *Administration of Antibodies*

Mice were administered 0.5 mL of either rabbit anti-MIP-1α or rabbit anti-MCP-1 intraperitoneally (ip) using a 25 gauge needle. The rabbit polyclonal antisera was specific for its respective chemokine and did not cross-react with other known chemokines or cytokines, as tested by ELISA. The sera had titers > $10^6$.

## RESULTS

### *Intragastric Administration of HGG Inhibits HGG-specific T-cell Proliferation and DTH*

Two groups of three mice were either given 20 mg HGG or myelin basic protein (MBP) (as a control) intragastrically (ig) seven days prior to immunization with 25 μg HGG emulsified in CFA containing 2 mg/mL *Mycobacterium tuberculosis*. Seven days following immunization, draining lymph node cells were pooled and cultured with HGG; proliferation was measured by thymidine incorporation. FIGURE 1 shows that draining lymph node cells from control mice fed MBP and immunized with HGG make a recall T cell-proliferative response (open squares). By contrast, draining lymph node cells from mice fed HGG have a significantly decreased recall T cell-proliferative response (open circles; $p < 0.01$, Student's *t* test) through a 2 log dose-response range.

To determine the effect of ig administration of HGG on *in vivo*, cell-mediated immune responses, we tested DTH in groups of mice fed either HGG or MBP (control) and subsequently immunized with HGG/CFA. Seven days following immunization, mice from both groups were tested for HGG-specific DTH. Prechallenge ear thickness was measured, 5 μg of HGG was injected into each ear, and 24 h later the increased ear swelling was measured as an indication of DTH. In FIGURE 2 oral administration of 20 mg HGG seven days prior to immunization with HGG/CFA resulted in a significant decrease ($p < 0.05$) in *in vivo* DTH responses compared to control (MBP)-fed mice.

### *Effect of Intragastric Administration of HGG on Chemokine and Cytokine Responses*

To test whether ig administration of HGG had an effect on the production of chemokines and cytokines, we fed mice either HGG or a control antigen (MBP) seven days prior to immunization with HGG/CFA. One week following immunization, draining lymph node cells from each group of mice were pooled and cultured in the presence or absence of HGG *in vitro*. The presence of chemokines and cytokines in the culture supernatants was

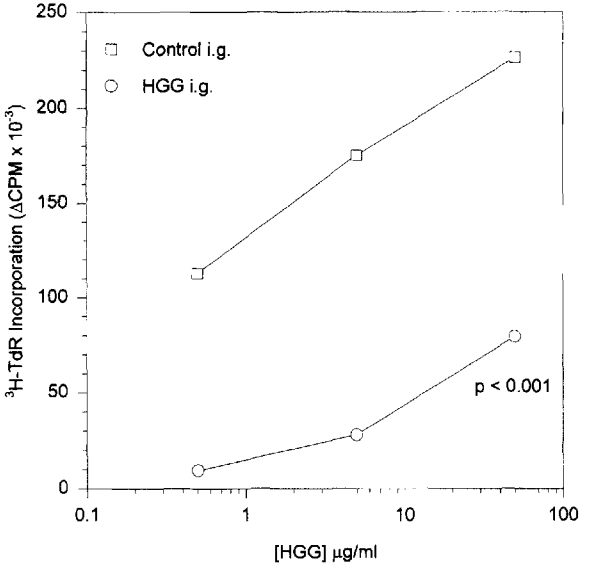


**FIGURE 1.** Intragastric administration of HGG decreases recall proliferative responses. Groups of three mice were fed 20 mg of either HGG or MBP (control) seven days prior to immunization with 25 μg HGG in CFA. Seven days later, draining lymph node cells were pooled and tested for proliferative responses in the presence or absence of 5 μg/mL HGG. The background proliferation for each group was less than 5000 cpm, and the standard deviation of each data point was less than 10% of the mean.

determined by specific ELISA.[36,46–48] Administration of ig HGG results in an antigen-specific decrease in IL-2 and IFN-γ, and an increase in IL-4 and IL-10 production (not shown), consistent with what has been reported in the literature.[49] In FIGURE 3 administration of 20 mg HGG ig also resulted in a decrease in MIP-1α production by lymph node cells from HGG-fed mice when compared to control-fed mice. However, to our surprise, ig administration of HGG had the opposite effect on production of MCP-1. FIGURE 4 shows that lymph node cells from mice fed 20 mg HGG 7 days prior to immunization produce a significantly greater amount of MCP-1. These results suggest that chemokines are important factors in the induction of oral tolerance.

### *Anti-MCP-1 Treatment* in Vivo *Abrogates Oral Tolerance Induction*

The differential recall production of MIP-1α (FIG. 3) and MCP-1 (FIG. 4) suggested that MCP-1 production *in vivo* might be important in the induction of oral tolerance. As a test of this hypothesis, we fed mice either HGG or ovalbumin (OVA; as a control) and treated ip with either normal rabbit serum (NRS) or antiserum to MIP-1α or MCP-1 on days 0 and 2, relative to feeding. Seven days after ig administration of antigen, all mice were immunized with HGG/CFA. One week following immunization, cells from the draining lymph nodes were pooled from the three mice in each group, and antigen-specific recall proliferation was measured. FIGURE 5 shows the result of this experiment. Cells

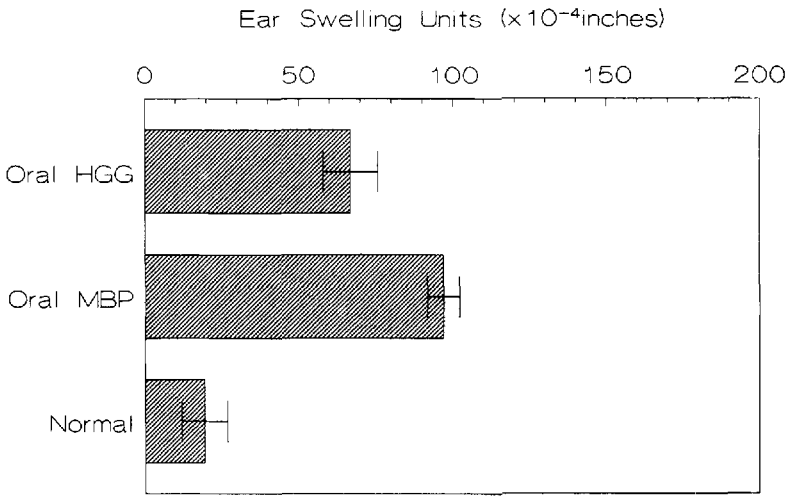


**FIGURE 2.** Intragastric administration of HGG results in decreased DTH responses. Mice were treated as in FIGURE 1. Seven days after immunization with HGG in CFA, the two groups of mice were challenged with 5 μg of HGG in PBS in each ear. Twenty-four hours later, ear swelling was used as a measure of DTH. The results are displayed as the mean ear swelling ± SD of three mice per group (6 ears). The DTH response in the oral HGG groups is statistically different ($p < 0.05$) than the DTH response for the control-fed group (oral MBP).

from mice fed OVA, treated with NRS, and immunized with HGG/CFA showed a substantial antigen-specific T cell-proliferative response (group A). By contrast, cells from mice fed HGG, treated with NRS, and immunized with HGG/CFA showed a significantly decreased proliferative response (group B; $p < 0.01$ when compared to group A). Cells from mice fed HGG, treated with anti-MIP-1α, and immunized with HGG/CFA (group D) also showed a decreased proliferative response when compared to its control group (group C; $p < 0.01$). The proliferative response of cells from group D is not significantly different than that from group B, suggesting that *in vivo* neutralization of MIP-1α does not have an effect on the induction of oral tolerance. However, cells from mice fed HGG, treated with anti-MCP-1, and immunized with HGG/CFA (group F) did not show a significantly different proliferative response than cells from the control-fed group (group E). Moreover, the proliferative response of the lymph node cells from mice fed HGG, treated with anti-MCP-1, and immunized with HGG-CFA (group F) was significantly greater ($p < 0.01$) than that of mice fed HGG and treated with either NRS (group B) or anti-MIP-1α (group D). These data suggest that induction of oral tolerance can be abrogated by treating recipients *in vivo* with anti-MCP-1, but not anti-MIP-1α.

## DISCUSSION

Feeding protein antigens can result in the antigen-specific inhibition of peripheral immune responses[50,51] as well as the priming of antigen-specific gut responses.[18] In the present report, we demonstrated that feeding a high dose of HGG can inhibit T cell-

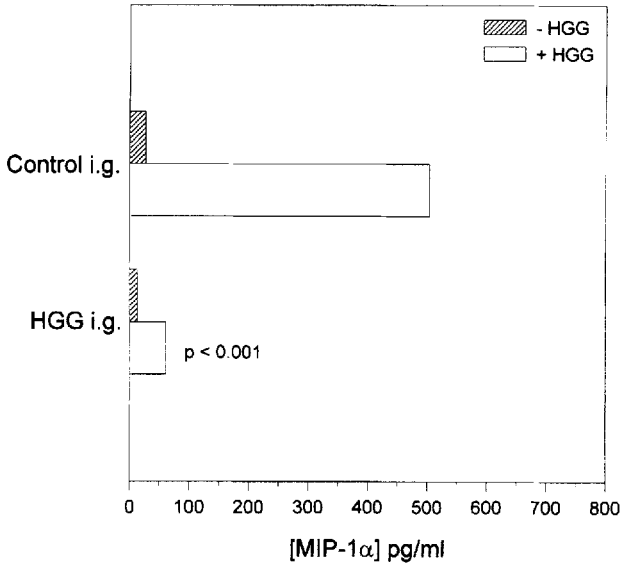


**FIGURE 3.** Intragastric (ig) administration of HGG results in the antigen-specific reduction in recall MIP-1α production *in vitro*. Lymph node cells from the mice in FIGURE 1 were cultured in the presence or absence of 5 μg/mL HGG. MIP-1α production was measured by ELISA, using recombinant MIP-1α as a standard, and the data are shown as pg/mL.

proliferative (FIG. 1) and DTH (FIG. 2) responses. Moreover, oral tolerance induction by feeding HGG selectively inhibited MIP-1α production (FIG. 3) and increased MCP-1 production (FIG. 4) by peripheral lymphoid cells. MCP-1 was found to be an important chemokine in the induction of oral tolerance because treatment with anti-MCP-1, but not MIP-1α, at the time of feeding HGG abrogated oral tolerance (FIG. 5). The mechanism or oral tolerance induction in the present report appears to be a combination of inhibition of Th1 and priming of Th2 cytokine responses (data not shown). Moreover, we have recently shown that feeding a high dose of the immunodominant peptide epitope of proteolipid protein results in peripheral tolerance by inducing both anergy of Th1 responses and priming of Th2 responses (manuscript submitted). Our results are consistent with what has been reported for inducing oral tolerance by feeding ovalbumin.[9,10]

One possibility that might explain why treatment with anti-MCP-1 would abrogate oral tolerance induction is that MCP-1 is an important factor in the generation of a Th2-like response after feeding HGG. We do not favor this interpretation because analysis of lymphocytes from mice treated with anti-MCP-1 revealed that there was no difference in IL-4 and TGF-β production by peripheral T cells when compared to T cells from control-treated mice (data not shown). Moreover, addition of either recombinant MCP-1 or MIP-1α to cultures of HGG-specific T cells did not alter the cytokine production pattern (data not shown). A more likely interpretation of the data in the present report is that MCP-1 is an important chemotactic factor in the gut and/or periphery and is selectively involved in the chemotaxis of Th2-like regulatory cells in and out of the gut, as well as the spleen. A recent report has suggested that MCP-1 production is induced in intestinal epithelial cells following infection.[45] It is possible that oral administration of antigens induces MCP-

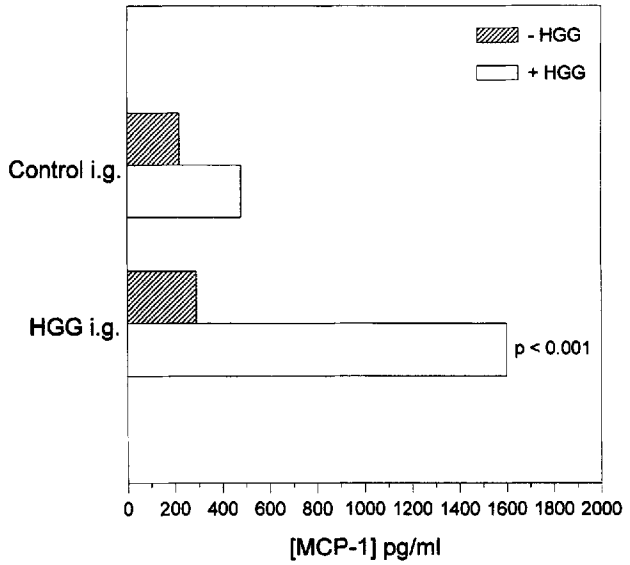


**FIGURE 4.** Intragastric (ig) administration of HGG results in the antigen-specific increase in recall MCP-1 production *in vitro*. Lymph node cells from the mice in FIGURE 1 were cultured in the presence or absence of 5 μg/mL HGG. MCP-1 production was measured by ELISA, using recombinant MCP-1 as a standard, and the data are shown as pg/mL.

1, which in turn induces the chemoattraction of Th2-like regulatory cells. By blocking the chemotactic effects of MCP-1 at the site of antigen intake, the influx of regulatory cells might be prevented, thereby preventing the induction of peripheral tolerance. Alternatively, MCP-1 might be involved in the efflux of regulatory cells from the gut to the periphery, and neutralization of MCP-1 in the gut would prevent the migration of these cells to the periphery. These possibilities are currently being tested.

In addition to MCP-1,[43] MIP-1α is also a chemotactic factor for T cells.[35] It is interesting that anti-MIP-1α did not abrogate the induction of oral tolerance (FIG. 5) in the present report. If the hypothesis is that oral tolerance induces a regulatory T-cell population that emigrates from the gut to the periphery to effect nonresponsiveness, one might predict that anti-MIP-1α would also abrogate induction of oral tolerance. We have previously shown that anti-MIP-1α treatment inhibits granuloma formation in both a schistosomiasis model[47] and a central nervous system demyelinating disease model, experimental autoimmune encephalomyelitis (EAE).[52] Both of these inflammatory disease models are induced by Th1-dominated responses. It is possible that MIP-1α is a preferential chemoattractant in Th1-dominated responses and that MCP-1 is a preferential chemoattractant in Th2-dominated responses. The data in the present report suggest that MCP-1 is an important chemotactic cytokine in the induction of oral tolerance, which has been reported to be mediated in part by a Th2-like response.[49]

## SUMMARY

Peripheral antigen-specific tolerance can be induced by feeding protein antigens. The mechanism has been described as either clonal anergy/deletion or induction of antigen-

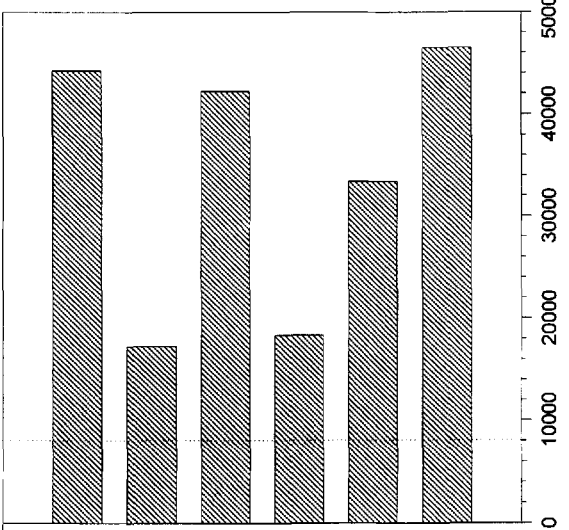


**FIGURE 5.** Anti-MCP-1 treatment abrogates oral tolerance induction. Six groups (A–F) of mice (3 per group) were fed either OVA (control) or HGG seven days prior to immunization and treated with either NRS, anti-MIP-1α, or anti-MCP-1 on days 0 and 2, relative to feeding. All mice were immunized with 25 μg HGG emulsified in CFA. Seven days after immunization, the draining lymph node cells were pooled from the three mice in each group, and recall proliferative responses were tested in the presence or absence of 5 μg/mL HGG. The results are displayed as mean thymidine incorporation (cpm) of triplicate wells. Statistical analysis was performed using ANOVA. The standard deviation was less than 10% of the mean in all groups and was left out of the FIGURE for clarity.

specific regulatory cells that produce transforming growth factor (TGF)-β, depending on the dose of antigen fed. Experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis, can be prevented by feeding myelin basic protein (MBP) or proteolipid protein (PLP). We decided to address the role of chemokines in the induction of oral tolerance. We have used a model antigen system of feeding a high dose of human gamma globulin (HGG) to mice that have been subsequently immunized with HGG emulsified in CFA. The result was decreased recall proliferative, delayed-type hypersensitivity (DTH) and Th1 cytokine responses. By contrast, Th2 cytokine responses were enhanced. Interestingly, macrophage inflammatory protein (MIP)-1α production was decreased, whereas monocyte chemotactic protein (MCP)-1 production was enhanced. Induction of oral tolerance was prevented by the administration of anti-MCP-1 to mice fed HGG. These results show that chemokines play an important role in the induction of oral tolerance.

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## REFERENCES

1. Weiner, H. L. 1994. Oral tolerance. Proc. Natl. Acad. Sci. USA **91:** 10762-10765.
2. Lider, O., L. M. Santos, C. S. Lee, P. J. Higgins & H. L. Weiner. 1989. Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein. II. Suppression of disease and *in vitro* immune responses is mediated by antigen-specific CD8+ T lymphocytes. J. Immunol. **142:** 748-752.
3. Bitar, D. M. & C. C. Whitacre. 1988. Suppression of experimental autoimmune encephalomyelitis by the oral administration of myelin basic protein. Cell. Immunol. **112:** 364-370.
4. Nussenblatt, R. B., R. R. Caspi, R. Mahdi, C.-C. Chan, F. Roberge, O. Lider & H. L. Weiner. 1990. Inhibition of S-antigen induced experimental autoimmune uveoretinitis by oral induction of tolerance with S-antigen. J. Immunol. **144:** 1689-1695.
5. Zhang, Z. Y., C. S. Lee, O. Lider & H. L. Weiner. 1990. Suppression of adjuvant arthritis in Lewis rats by oral administration of type II collagen. J. Immunol. **145:** 2489-2493.
6. Wang, Z.-Y., J. Huang, T. Olsson, B. He & H. Link. 1995. B cell responses to acetylcholine receptor in rats orally tolerized against experimental autoimmune myasthenia gravis. J. Neurol. Sci. **128:** 167-174.
7. Weiner, H. L., G. A. Mackin, M. Matsui, E. J. Orav, S. J. Khoury, D. M. Dawson & D. A. Hafler. 1993. Double-blind pilot trial of oral tolerance with myelin antigens in multiple sclerosis. Science **259:** 1321-1323.
8. Trentham, D. E., R. A. Dynesius-Trentham, E. J. Orav, D. Combitchi, C. Lorenzo, K. L. Sewell, D. A. Hafler & H. L. Weiner. 1993. Effects of oral administration of type II collagen on rheumatoid arthritis Science **261:** 1727-1730.
9. Miller, S. D. & D. G. Hanson. 1979. Inhibition of specific immune responses by feeding protein antigens. IV. Evidence for tolerance and specific active suppression of cell-mediated immune responses to ovalbumin. J. Immunol. **123:** 2344-2350.
10. Hanson, D. G. & S. D. Miller. 1982. Inhibition of specific immune responses by feeding protein antigens. V. Induction of the tolerant state in the absence of specific suppressor cells. J. Immunol. **128:** 2378-2381.
11. Asherson, G. L., M. Zembala, M. A. C. C. Perera, B. Mayhew & W. R. Thomas. 1977. Production of immunity and unresponsiveness in the mouse by feeding contact

sensitizing agents and the role of suppressor cells in the Peyer's patches, mesenteric lymph nodes and other lymphoid tissues. Cell. Immunol. **33:** 145-155.

12. STROBEL, S., A. M. MOWAT, H. E. DRUMMOND, M. G. PICKERING & A. FERGUSON. 1983. Immunological responses to fed protein antigens in mice. Oral tolerance for CMI is due to activation of cyclophosphamide-sensitive cells by gut-processed antigen. Immunology **49:** 451-456.
13. RICHMAN, L. K., J. M. CHILLER, W. R. BROWN, D. G. HANSON & N. M. VAZ. 1978. Enterically induced immunologic tolerance. I. Induction of suppressor T lymphocytes by intragastric administration of soluble proteins. J. Immunol. **121:** 2429-2434.
14. WHITACRE, C. C., I. E. GIENAPP, C. G. OROSZ & D. M. BITAR. 1991. Oral tolerance in experimental autoimmune encephalomyelitis. III. Evidence for clonal anergy. J. Immunol. **147:** 2155-2163.
15. MILLER, A., O. LIDER, A. B. ROBERTS, M. B. SPORN & H. L. WEINER. 1992. Suppressor T cells generated by oral tolerization to myelin basic protein suppress both *in vitro* and *in vivo* immune responses by the release of transforming growth factor β after antigen-specific triggering. Proc. Natl. Acad. Sci. USA **89:** 421-425.
16. FRIEDMAN, A. & H. L. WEINER. 1994. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. Proc. Natl. Acad. Sci. USA **91:** 6688-6692.
17. KAY, R. A. & A. FERGUSON. 1989. The immunological consequences of feeding cholera toxin. II. Mechanisms responsible for the induction of oral tolerance for DTH. Immunology **66:** 416-421.
18. MICHALEK, S. M., J. R. MCGHEE, H. KIYONO, D. E. COLWELL, J. H. ELDRIDGE, M. J. WANNEMUEHLER & W. J. KOOPMAN. 1983. The IgA response: Inductive aspects, regulatory cells, and effector functions. Ann. N.Y. Acad. Sci. **409:** 48-71.
19. ENOMOTO, A., M. KONISHI, S. HACHIMURA & S. KAMINOGAWA. 1993. Milk whey protein fed as a constituent of the diet induced both oral tolerance and a systemic humoral response, while heat-denatured whey protein induced only oral tolerance. Clin. Immunol. Immunopathol. **66:** 136-142.
20. HUSBY, S., J. MESTECKY, Z. MOLDOVEANU, S. HOLLAND & C. O. ELSON. 1994. Oral tolerance in humans. T cell but not B cell tolerance after antigen feeding. J. Immunol. **152:** 4663-4670.
21. ELSON, C. O. & W. EALDING. 1984. Cholera toxin feeding did not induce oral tolerance in mice and abrogated oral tolerance to an unrelated protein antigen. J. Immunol. **133:** 2892-2897.
22. FARIA, A. M., G. GARCIA, M. J. RIOS, C. L. MICHALAROS & N. M. VAZ. 1993. Decrease in susceptibility to oral tolerance induction and occurrence of oral immunization to ovalbumin in 20- to 38-week-old mice. The effect of interval between oral exposures and rate of antigen intake in the oral immunization. Immunology **78:** 147-151.
23. HANSON, D. G., M. J. ROY, G. M. GREEN & S. D. MILLER. 1993. Inhibition of orally-induced immune tolerance in mice by prefeeding an endopeptidase inhibitor. Reg. Immunol. **5:** 85-93.
24. MICHALEK, S. M., H. KIYONO, M. J. WANNEMUEHLER, L. M. MOSTELLER & J. R. MCGHEE. 1982. Lipopolysaccharide (LPS) regulation of the immune response: LPS influence on oral tolerance induction. J. Immunol. **128:** 1992-1998.
25. RUSSEL, G. & C. COSTALOS. 1980. Oral tolerance of Caloreen in babies. Arch. Dis. Child. **55:** 886-887.
26. DOMEN, P. L., A. MUCKERHEIDE & J. G. MICHAEL. 1987. Cationization of protein antigens. III. Abrogation of oral tolerance. J. Immunol. **139:** 3195-3198.
27. SUN, J.-B., J. HOLMGREN & C. CZERKINSKY. 1994. Cholera toxin B subunit: An efficient transmucosal carrier-delivery system for induction of peripheral immunological tolerance. Proc. Natl. Acad. Sci. USA **91:** 10795-10799.
28. VAJDY, M., M. H. KOSCO-VILBOIS, M. KOPF, G. KÖHLER & N. LYCKE. 1995. Impaired mucosal immune responses in interleukin 4-targeted mice. J. Exp. Med. **181:** 41-53.

29. SUZUKI, I., H. KIYONO, K. KITAMURA, D. R. GREEN & J. R. MCGHEE. 1986. Abrogation of oral tolerance by contrasuppressor T cells suggests the presence of regulatory T-cell networks in the mucosal immune system. Nature **320:** 451-454.
30. SUH, E. D., B. P. VISTICA, C. C. CHAN, J. M. RABER, I. GERY & R. B. NUSSENBLATT. 1993. Splenectomy abrogates the induction of oral tolerance in experimental autoimmune uveoretinitis. Curr. Eye Res. **12:** 833-839.
31. SCHALL, T. J. 1991. Biology of the RANTES/SIS cytokine family. Cytokine **3:** 165-183.
32. MILLER, M. D. & M. S. KRANGEL. 1992. Biology and biochemistry of the chemokines: a family of chemotactic and inflammatory cytokines. Crit. Rev. Immunol. **12:** 17-46.
33. OPPENHEIM, J. J., C. O. C. ZACHARIAE, N. MUKAIDA & K. MATSUSHIMA. 1991. Properties of the novel proinflammatory supergene "intercrine" cytokine family. Annu. Rev. Immunol. **9:** 617-648.
34. ERNST, C. A., Y. J. ZHANG, P. R. HANCOCK, B. J. RUTLEDGE, C. L. CORLESS & B. J. ROLLINS. 1994. Biochemical and biologic characterization of murine monocyte chemoattractant protein-1. J. Immunol. **152:** 3541-3549.
35. DAVATELIS, G., P. TEKAMP-OLSON, S. D. WOLPE, K. HERMSEN, C. LUEDKE, C. GALLEGOS, D. COIT, J. MERRYWEATHER & A. CERAMI. 1988. Cloning and characterization of a cDNA for murine macrophage inflammatory protein (MIP), a novel monokine with inflammatory and chemokine properties. J. Exp. Med. **167:** 1939-1944.
36. LUKACS, N. W., S. W. CHENSUE, R. E. SMITH, R. M. STRIETER, K. WARMINGTON, C. WILKE & S. L. KUNKEL. 1994. Production of monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 alpha by inflammatory granuloma fibroblasts. Am. J. Pathol. **144:** 711-718.
37. BUTCHER, E. C. 1991. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. Cell **67:** 1033-1036.
38. SPRINGER, T. A. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell **76:** 301-314.
39. VADDI, K. & R. C. NEWTON. 1994. Regulation of monocyte integrin expression by β-family chemokines. J. Immunol. **153:** 4721-4732.
40. TANAKA, Y., D. H. ADAMS, S. HUBSCHER, H. HIRANO, U. SIENBENLIST & S. SHAW. 1993. T-cell adhesion induced by proteoglycan-immobilized cytokine MIP-1β. Nature **361:** 79-82.
41. ROLLINS, B. J., E. D. MORRISO & C. D. STILES. 1988. Cloning and expression of JE, a gene inducible by platelet-derived growth factor and whose product has cytokine-like properties. Proc. Natl. Acad. Sci. USA **85:** 3738-3742.
42. VAN DAMME, J., P. PROOST, W. PUT, S. ARENS, J.-P. LENAERTS, R. CONINGS, G. OPDENAKKER, H. HEREMANS & A. BILLIAU. 1994. Induction of monocyte chemotactic proteins MCP-1 and MCP-2 in human fibroblasts and leukocytes by cytokines and cytokine inducers. Chemical synthesis of MCP-2 and development of a specific RIA. J. Immunol. **152:** 5495-5502.
43. CARR, M. W., S. J. ROTH, E. LUTHER, S. S. ROSE & T. A. SPRINGER. 1994. Monocyte chemoattractant protein 1 acts as a T-lymphocyte chemoattractant. Proc. Natl. Acad. Sci. USA **91:** 3652-3656.
44. LOETSCHER, P., M. SEITZ, I. CLARK-LEWIS, M. BAGGIOLINI & B. MOSER. 1994. Monocyte chemotactic proteins MCP-1, MCP-2, and MCP-3 are major attractants for human $CD4^+$ and $CD8^+$ T lymphocytes. FASEB J. **8:** 1055-1060.
45. JUNG, H. C., L. ECKMANN, S.-K. YANG, A. PANJA, J. FIERER, E. MORZYCKA-WROBLEWSKA & M. F. KAGNOFF. 1995. A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. J. Clin. Invest. **95:** 55-65.
46. PETERSON, J. D., W. J. KARPUS, R. J. CLATCH & S. D. MILLER. 1993. Split tolerance of Th1 and Th2 cells in tolerance to Theiler's murine encephalomyelitis virus. Eur. J. Immunol. **23:** 46-55.

47. LUKACS, N. W., S. L. KUNKEL, R. M. STRIETER, K. WARMINGTON & S. W. CHENSUE. 1993. The role of macrophage inflammatory protein 1α in Shistosoma mansoni egg-induced granulomatous inflammation. J. Exp. Med. **177:** 1551-1559.
48. KARPUS, W. J., J. D. PETERSON & S. D. MILLER. 1994. Anergy *in vivo:* down-regulation of antigen-specific $CD4^+$ Th1 but not Th2 cytokine responses. Int. Immunol. **6:** 721-730.
49. CHEN, Y., V. K. KUCHROO, J. INOBE, D. A. HAFLER & H. L. WEINER. 1994. Regulatory T cell clones induced by oral tolerance: Suppression of autoimmune encephalomyelitis. Science **265:** 1237-1240.
50. TOMASI, T. B., JR. 1980. Oral tolerance. Transplantation **29:** 353-356.
51. WEINER, H. L., A. FRIEDMAN, A. MILLER, S. J. KHOURY, A. AL-SABBAGH, L. SANTOS, M. SAYEGH, R. B. NUSSENBLATT, D. E. TRENTHAM & D. A. HAFLER. 1994. Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. Annu. Rev. Immunol. **12:** 809-837.
52. KARPUS, W. J., N. W. LUKACS, B. L. MCRAE, R. M. STRIETER, S. L. KUNKEL & S. D. MILLER. 1995. An important role for the chemokine macrophage inflammatory protein-1α in the pathogenesis of the T-cell mediated autoimmune disease, experimental autoimmune encephalomyelitis. J. Immunol. **155:** 5003-5010.