Oral tolerance: A new tool for the treatment of gastrointestinal inflammatory disorders and liver-directed gene therapy

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Y Ilan. Oral tolerance: A new tool for the treatment of gastrointestinal inflammatory disorders and liver-directed gene therapy. Can J Gastroenterol 1999;13(10):829-835. Oral tolerance is a method of downregulating an immune response by feeding antigens. The use of oral tolerance toward adenoviruses and colitis-extracted proteins for long term gene therapy and alleviation of experimental colitis, and the mechanisms of tolerance induction are presented. Adenoviruses are efficient vectors in liver-directed gene therapy; however, the antiviral immune response precludes the ability to achieve long term gene expression and prohibits the ability to reinject the recombinant virus. Oral tolerance induction via feeding of viral-extracted proteins prevented the anti-adenoviral humoral and cellular immune responses, thus enabling long term gene therapy using these viruses. Moreover, pre-existing immune response to the virus was overcome by tolerance induction, enabling prolonged gene expression in a presensitized host. Inflammatory bowel diseases are immune-mediated disorders where an imbalance between proinflammatory (T helper cell type 1) and anti-inflammatory (T helper cell type 2) cytokines are thought to play a role in the pathogenesis. In the experimental colitis model, the feeding of colitis-extracted proteins downregulated the anticolon immune response. Tolerance induction toward colitis-extracted proteins ameliorated colonic inflammation as shown by decreased diarrhea and reduction of colonic ulcerations, intestinal and peritoneal adhesions, wall thickness and edema. Histological parameters for colitis were markedly improved in tolerized animals. In both models, tolerized animals developed an increase in transforming growth factor-beta, interleukin-4 and interleukin-10, and a decrease in the mRNA of interferon-gamma lymphocytes and serum levels. Adoptive transfer of tolerized lymphocytes enabled the transfer of tolerance toward adenoviruses and colon-extracted proteins. Thus, oral tolerance induces suppressor lymphocytes that mediate immune response downregulation by induction of a shift from a proinflammatory T helper cell type 1 to an anti-inflammatory T helper cell type 2 immune response.

Key Words: *Adenovirus; Inflammatory bowel disease; Oral tolerance*

Tolérance orale : Nouvel outil pour le traitement des maladies inflammatoires gastro-intestinales et pour la thérapie génique hépatique

La tolérance orale est une méthode par laquelle on vise à atténuer la réponse immunitaire par l'administration d'antigènes. On aborde ici l'emploi de la tolérance orale à l'encontre d'adénovirus et de protéines extraites de spécimens de colite pour une thérapie génique à long terme et le soulagement de la colite induite expérimentalement, et les mécanismes de l'induction de la tolérance. Les adénovirus sont des vecteurs efficaces pour la thérapie génique dirigée au foie. Par contre, la réponse immunitaire antivirale empêche l'expression génique à long terme et la réinjection du virus recombinant. L'induction de la tolérance orale par le biais de l'administration de protéines extraites de virus a empêché les réponses anti-adénovirales humorales et cellulaires, ce qui permet une thérapie

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This mini-review was prepared from a presentation made at the World Congress of Gastroenterology, September 6 to 11, 1998, Vienna, Austria
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Received for publication November 2, 1998. Accepted November 9, 1998

génique à long terme à partir de ces virus. De plus, la réaction immunitaire pré-existante au virus a été surmontée par l'induction de la tolérance, ce qui permet une expression génique prolongée chez un hôte préalablement sensibilisé. Les maladies inflammatoires de l'intestin sont des troubles à médiation immunitaire, caractérisés par un déséquilibre entre les cytokines pro-inflammatoires (lymphocytes T de type 1) et anti-inflammatoires (lymphocytes T de type 2) dont on pense qu'elles pourraient jouer un rôle pathogène. Dans un modèle de colite induite expérimentalement, l'administration de protéines extraites de spécimens de colite a permis d'atténuer la réaction immunitaire dirigée contre le côlon. L'induction de la tolérance à l'endroit des protéines extraites de spécimens de colite a amélioré les signes d'inflammation colonique comme en fait foi une réduction de la diarrhée et des ulcérations coloniques, des adhésions intestinales et péritonéales, de l'épaisseur de la paroi et de l'œdème. Les paramètres histologiques de la colite ont été nettement améliorés chez les animaux où on avait induit la tolérance. Dans les deux modèles, ces animaux ont manifesté une transformation accrue du facteur bêta, de l'interleukine 4 et de l'interleukine 10 et une diminution de l'ARNm des lymphocytes de l'interféron gamma et des taux sériques. Le transfert des lymphocytes « tolérés » a permis le transfert de la tolérance vers les adénovirus et vers les protéines extraites des spécimens de côlon. Ainsi, la tolérance orale provoque les lymphocytes suppresseurs qui influent sur l'atténuation de la réponse immunitaire par l'induction d'une réponse immunitaire qui ne dépend plus des lymphocytes T de type 1 pro-inflammatoires, mais bien des lymphocytes de type 2 anti-inflammatoires.

Oral tolerance is the induction of immunological hyporesponsiveness through oral administration of the antigen (1). The first description of lymphoid structures in the gut was published in 1677 by Peyer. In 1911, Wells (2) fed hen proteins to chickens, which were then found to be resistant to anaphylaxis when challenged. In 1946, Chase (3) published the observation that guinea pigs fed a contact-sensitizing agent showed a decreased skin reactivity to this agent. Over the past 15 years, major progress has been achieved by several groups for the use of oral tolerance in the treatment of animal and human models of autoimmune disorders (4). Induction of oral tolerance has been shown to prevent or alleviate immune-mediated disorders in animals, such as collagen-induced arthritis, experimental allergic encephalomyelitis, systemic sclerosis, contact dermatitis, diabetes mellitus and myasthenia gravis (5-8). Oral tolerance has been used in humans for the treatment of several autoimmune diseases such as rheumatoid arthritis, diabetes mellitus, uveitis, thyroiditis and multiple sclerosis (1,9-11).

MECHANISMS OF ORAL TOLERANCE

Although the oral feeding of an antigen is well established as a method of immune tolerance induction, the exact mechanism has yet to be discovered. Antigen presented to gut epithelial cells, intraepithelial lymphocytes or M cells are processed and may even be absorbed through the portal circulation, leading to re-education of the immune system (1,12,13). Immune tolerance was traditionally divided into two categories: central tolerance, where tolerance is induced by presentation of foreign antigens in the thymus, and peripheral tolerance, where tolerance is induced outside the thymus (14,15). The current practice is to divide immune tolerance into 'dominant' and 'passive' types of tolerance based on the mechanism of tolerance induction. A 'dominant' type of tolerance involves the production of suppressor cells that suppress the antiforeign antigen immune response and can be transferred. The 'passive' type of immune tolerance involves immune ignorance or clonal deletion and is nontransferable (14,15). Oral tolerance can induce both types of tolerance. Feeding low antigen doses induces tolerance via the generation of suppressor cells, while feeding higher doses leads to deletion of immune-reactive cells, clonal inactivation or anergy (1,16). Both the humoral and the cellular limbs of the immune response are affected by oral tolerance. Another important mechanism is a bystander effect achieved by feeding closely related antigens that induce an immune-suppressed state toward the target antigen (1,12). As regulatory cells secrete nonantigen-specific cytokines, after being triggered by a fed antigen, they suppress inflammation in the microenvironment where the fed antigen is localized. Clinical trials performed in humans with various immune-mediated disorders use bystander antigens for this purpose (8-11). However, several of these studies showed only partial or no effect of oral tolerance (17). In these studies the orally administered antigens were related but not identical to the actual antigenic target of the autoimmune disease. Specificity of the antigen regulates its uptake and presentation patterns at the levels of gut epithelial or other antigen-presenting cells of the gut-associated lymphoid tissue. Thus, the lack of specificity of the fed antigens may have been responsible for these failures (18,19). Conflicting results as to whether an antigen has to be processed and/or absorbed, and whether protein denaturation is necessary for tolerance induction have been published (20). Intravenous administration of denatured proteins induced tolerance similar to that produced by the feeding of whole native proteins (20). Antigen presentation may require whole proteins to be presented into the bowel; however, protein processing and absorption may also be involved in tolerance induction or in its maintenance through postgut mechanisms (21). Gut wall epithelial cells, Peyer's patches, mesenteric lymph node or extraintestinal cells have been suggested to be mediators of immune tolerance induction (21). However, oral administration of an antigen can also elicit an epitope-specific immunity (18,19). Side by side with immunosuppressive cytokine-secreting cells (eg, transforming growth factor-beta [TGF-β]) that appear after oral tolerization, a second population of cells, secreting pro-inflammatory cytokines (eg, interferon-gamma [IFN-γ]) exist in the gut wall, mainly in Peyer's patches (22,23). Thus, orally administered antigen elicits a local pro-inflammatory, IFN-γ-mediated response in the gut mucosa, along with a systemic TGF-β and interleukin (IL)-4-mediated anti-inflammatory response (12,13). The induction of oral tolerance requires a balance between an

immunogenic and a tolerogenic cell population, with a shift from T helper cell type 1 (Th1) (and secretion of pro-inflammatory cytokines) to a T helper cell type 2 (Th2) (and secretion of anti-inflammatory cytokines) immune response.

USE OF ORAL TOLERANCE IN GENE THERAPY USING RECOMBINANT ADENOVIRAL VECTORS

The two major requirements for successful gene therapy are achievement of an adequate level of transgene expression and long term expression of the gene. Recombinant adenoviruses have many advantages for use as vectors in gene therapy (24). They have a high efficiency of gene transfer into nondividing cells, and have preferred localization and expression in the liver, making them ideal vectors for use in gene therapy for liver disorders (25-27). These viruses are nononcogenic and exhibit a low level of pathogenicity in humans. On the other hand, transgene expression is of limited duration because the virus is episomal. In addition, host immune response further limits transgene expression and precludes expression after reinjection of the virus (28-30). Virus readministration fails to produce transgene expression because of host humoral (neutralizing antibodies) and cellular (cytotoxic lymphocytes [CTL]) immune responses (28). Moreover, most adult humans have pre-existing neutralizing antibodies against adenoviruses, which pose a major obstacle to clinical application of these vectors (24). After initial injection of the virus, antibodies against viral antigens and CTLs against virally infected cells are produced (31). The antibody titres gradually decline, but memory cells persist. Although the antibodies are neutralizing, once the titres fall, it is possible to reinfect the animal with a second injection of the virus (28-29). This results, however, in a booster response, creating a much higher titre of the antibodies and a marked CTL response, resulting in hepatic infiltration of these cells, rapid clearance of the virus and failure of any beneficial effect of transgene expression. Major histocompatibility complex (MHC) class I-restricted CD8+ host lymphocytes are important effectors of destruction of hepatocytes infected by recombinant adenoviruses (28,29). Systemic delivery of recombinant adenoviruses activates viral antigen-specific responses of both MHC class I-restricted CTLs and MHC class II-restricted Th1 cells (28). Studies in beta-microglobulin knockout mice suggest that MHC class I-restricted CD4+ cells may also destroy adenovirus-infected hepatocytes in vitro (28-30).

Oral tolerance was recently described as a method to overcome the antiadenoviral immune response (32). The Gunn rat was used as the model for gene therapy for metabolic disorders in these studies. This rat is a genetic and metabolic model of Crigler Najjar type I. Inherited deficiency of bilirubin-UDP-glucuronosyl transferase (BUGT) causes Crigler Najjar syndrome type I (33). In patients with this syndrome, severe unconjugated hyperbilirubinemia results in potentially lethal brain damage. Liver transplantation is the only definitive treatment (34). The recombinant adenovirus used in these studies expresses the human BUGT gene driven by a cytomegalovirus promoter (31). The recombinant virus was prepared by disruption of the E_1 region of the wild-type human adenovirus type 5, by cloning the transgene into the E_1 region, thus leading to a replicative defective virus expressing the human BUGT gene.

To test whether the host immune response can be abrogated by enteral administration of adenoviral antigens, Gunn rats were given 10 low doses of adenoviral protein extracts through a gastrojejunostomy tube on alternate days for three weeks. Control rats were fed with the same dose of bovine serum albumin. After completion of the enteral protein administration, 5×10^9 plaque forming units (pfu) of a recombinant adenovirus expressing either human $BUGT_1$ (Ad-h$BUGT_1$) or *Escherichia coli* beta-galactosidase (Ad-LacZ) was injected intravenously on days 1 and 98. The rats were evaluated for gene expression by polymerase chain reaction (PCR), Western blots, serum bilirubin levels, bile analysis by high performance liquid chromatography and liver enzyme activity, and for the humoral and cellular antiadenovirus immune response (32).

In the control group, serum bilirubin levels decreased after the first injection, but the effect was transient. In this group, subsequent injection failed to reduce serum bilirubin levels again. In contrast, in rats fed with viral proteins, serum bilirubin levels were markedly reduced for a longer period after the first virus injection. A second injection led to reinduction of the hypobilirubinemic effect. $BUGT_1$ was expressed in the liver, and serum bilirubin levels were reduced from 6.9 to 2.1 mg/dL in one week. After two months, serum bilirubin increased gradually; a second dose of the virus on day 98 markedly reduced serum bilirubin levels again. A subsequent injection of Ad-LacZ led to positive beta-galactosidase staining in more than 90% of hepatocytes. The majority of the rats in the orally tolerized group did not develop detectable levels of antiadenovirus antibodies. In those that did, the antibody titres were low enough not to interfere with gene expression after reinjection. In contrast, all control rats developed high titres of the neutralizing antibodies. CTLs can clear virally infected cells after the first injection of the virus. The cellular immune response was markedly downregulated in the tolerized rats. In contrast, marked killing activity was observed when control lymphocytes were tested. Liver biopsies taken from control nontolerized rats following two recombinant adenovirus injections showed severe intrahepatic lymphocytic infiltration. In contrast, almost completely normal liver biopsies were observed in orally tolerized rats. Serum $TGF\beta_1$ levels were increased in the tolerized rats, and $TGF\beta_1$ was produced by lymphocytes from these rats but not from controls on exposure to the viral antigens in vitro. In addition, the expression of IL-2, IL-4 and IL-10 mRNAs by these lymphocytes was increased, whereas IFN-γ expression became undetectable. Thus, oral tolerization with low dose adenoviral antigen extracts permits long term gene expression after repeated adenovirus-directed gene transfer and may be useful in adenovirus-mediated gene therapy (32). This mode of tolerance induction was found to be superior to other methods

used for induction of tolerance toward viruses (35-37). Furthermore, as demonstrated for a variety of oral tolerance models, this suppression was associated with the development of antigen-specific T cells, secreting Th2 cytokines and TGFβ_1. In addition, there was a marked downregulation of Th1 cytokine production (1,12). The liver was recently found to play a major role in oral tolerance induction toward adenoviruses (38).

LONG TERM GENE THERAPY IN THE PRESENCE OF PRE-EXISTING ANTIADENOVIRAL IMMUNE RESPONSE

Infection by adenovirus type 5, which is used most commonly for the construction of gene transfer vectors, is common in humans (24). Therefore, many adult subjects have pre-existing neutralizing antibodies and CTLs against adenoviruses. Injection of a recombinant adenovirus in these subjects would be expected to produce a strong secondary immune response, resulting in rapid clearance of the virus and CTL invasion of the tissues infected by the recombinant virus. Therefore, we wanted to determine whether oral tolerization can prevent the secondary 'booster' response evoked by the administration of an adenoviral vector into an animal with pre-existing antiadenoviral immunity (39).

To test whether pre-existing antiviral antibodies can be downregulated by enteral administration of adenoviral antigens, $BUGT_1$-deficient jaundiced Gunn rats were immunized with a recombinant adenovirus (5×10^9 pfu/rat) expressing the human $BUGT_1$ gene (Ad-h$BUGT_1$). All rats developed cellular and humoral immune responses. Following this injection, mean serum bilirubin levels were reduced from 7.0 mg/dL to 2.3 mg/dL in seven days and then progressively increased to pretreatment levels in six weeks. All recipients developed high titre ($1:2^{15}$) neutralizing antibodies. To reduce the antibody titre, low dose protein extracts of a recombinant adenovirus type 5 were instilled via gastroduodenostomy tubes 10 to 40 days after the initial virus injection; control rats received bovine serum albumin (BSA). A second AdhBUGT_1 injection was given 72 days after the first injection. All the control rats, fed with BSA, developed higher antiviral antibody titres following the second virus injection. In contrast, in the tolerized rats, fed with viral proteins, the antiadenovirus humoral immune response was downregulated. Their antibody titres gradually decreased to $1:2^4$ and were low enough not to interfere with gene expression after virus reinjection. Moreover, in the tolerized rats, virus reinjection did not lead to secondary humoral immune response. The cellular immune response was downregulated in the tolerized rats fed with the viral proteins. In contrast, marked killing activity was observed when lymphocytes from the controlled nontolerized rats were tested. Expression of the hBUGT gene in previously immunized rats was measured by testing serum bilirubin levels at various time points after virus injections. In the control group, injected with the recombinant virus expressing the human BUGT gene and fed with BSA, serum bilirubin levels decreased after the first injection, but the effect was transient. Subsequent injection in this group failed to reduce serum bilirubin levels again. In contrast, in rats injected with a recombinant virus and fed the viral protein extract, serum bilirubin levels were reduced after the first virus injection, and a second injection led to reinduction of the hypobilirubinemic effect. Lymphocytes from the tolerized rats expressed TGF-β on exposure to antigen-presenting cells primed with adenoviral antigens, whereas IFN-γ expression was abolished. In contrast, lymphocytes from the BSA-treated rats overexpressed IFN-γ, and TGF-β_1 expression was undetectable. In the adenovirus-fed tolerized rats, but not in the BSA-treated controls, the second Ad-h$BUGT_1$ injection induced hepatic h$BUGT_1$ expression, resulting in a hypobilirubinemic response similar to that seen with the first injection. These studies showed that tolerization to recombinant adenoviruses by enteral administration of the major adenoviral structural proteins into preimmunized rats prevents the secondary booster response. This permits repeated adenovirus-directed gene transfer, despite the presence of a residual antibody titre from a previous exposure to the virus (39). This effect was mediated by suppressor cells that secrete immunoregulatory cytokines in an antigen-specific manner.

MECHANISM OF ORAL TOLERIZATION TO ADENOVIRAL VECTORS: DETERMINING THE ROLE OF NEGATIVE IMMUNOMODULATORY CELLS IN TOLERANCE INDUCTION

The tolerization methods described above may involve clonal anergy, clonal deletion or proliferation of negative immunoregulatory cells. To evaluate whether negative immunoregulatory lymphocytes are important in these tolerization procedures, we studied adoptive transfer from all groups of orally tolerized donors (23). Gut or splenic lymphocytes harvested from tolerized rats were infused into irradiated naive Gunn rats. Subsequent injection of the recombinant virus showed that only splenic lymphocytes, but not the gut lymphocytes, mediated the adoptive transfer of tolerance. Therefore, we determined the cytokine expression pattern of rat splenocytes after in vitro virus stimulation by reverse transcription PCR. Splenocytes from orally tolerized rats but not from naive control rats expressed TGF-β as well as IL-4 and IL-10. Serum TGF-β levels were also elevated in these rats. In contrast, IFN-γ was downregulated in splenocytes from tolerized rats. In nontolerized controls, high levels of IFN-γ secretion were observed. These results suggest that ingestion of low dose antigens leads to activation of suppressor cells in the systemic immune system and then to secretion of immunosupressive cytokines. These cytokines may be responsible for the induction of tolerance. Low dose oral tolerance led to the induction of a 'dominant' type of tolerance (23). Suppressor lymphocytes were able to transfer the antiviral tolerance when harvested from these animals and transplanted into naive irradiated animals. The suppressor cells were found to be present in the spleens of tolerized animals but not in the gut wall. This is consistent with the hypothesis that the memory for the tolerance resides

in the spleen or other lymphoid organs, while the bowel cells serve as a vehicle for antigen presentation, processing and educating the suppressor regulatory cells (23).

USE OF ORAL TOLERANCE FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASES

Inflammatory bowel diseases are common disorders of the gastrointestinal tract, where the immune response plays a major role in the pathogenesis. Mucosal antigens exposed through toxic, infectious or other mechanisms are targets for immune-mediated damage (40-42). An imbalance between Th1 proinflammatory and Th2 anti-inflammatory cytokines, secreted by activated T cells, is important in the pathogenesis of these disorders. In both animals and humans, tolerance induction is associated with a Th2-type immune response, leading to the secretion of immunosuppressive cytokines such as TGF-β_1, IL-4 and IL-10 (43-45). Tolerance induction was used in an animal model of experimental colitis (8). The aims of these studies were to test whether induction of oral tolerance toward colitis-extracted proteins can alleviate experimental colitis and to evaluate whether the transfer of suppressor cells can induce immune tolerance. These studies used a model system that employs normal rats treated with 2,4,6-trinitrobenzene sulphonic acid (TNBS). This material induces an autoimmune response resembling Crohn's disease in humans (8). This type of experimental colitis, similar to the human disease, is a Th1-mediated immune disorder resulting in life-long inflammatory response against the colon. Recently it was shown that TNBS-induced colitis could be prevented by TGF-β-mediated oral tolerance in mice fed a mixture of colonic proteins exposed to TNBS in vitro (haptenized colonic proteins) (8). However, in these studies, only the preventive role of oral tolerance was documented, and the ability to treat active colitis by tolerance induction was not shown. Therefore, we sought to evaluate whether induction of oral tolerance toward colitis-extracted proteins could be used to abrogate the host anticolon immune response for treating experimental colitis.

Colitis was induced by rectal instillation of TNBS. Treated rats were fed five oral doses of colitis-extracted proteins every other day. Control rats were fed the vehicle alone. Rats were followed for clinical, macroscopic and microscopic manifestations of colitis. Induction of oral tolerance toward colitis-extracted proteins alleviated experimental colitis. Oral tolerization toward colitis-extracted proteins alleviates experimental colitis (46). A marked decrease in diarrhea was observed in tolerized rats compared with nontolerized controls throughout the study period. The percentage of colonic area exhibiting macroscopic injury decreased to 57% in tolerized rats versus 72% in controls (P=0.14), and the mean colon weight was 1.6 g compared with 2.2 g in controls (P=0.02). In tolerized rats, degree of colonic ulceration, intestinal and peritoneal adhesions, and wall thickness improved significantly (P<0.05). The total score of all macroscopic parameters tested was 1.01 in tolerized rats compared with 2.11 in nontolerized controls (P=0.001). Histological evaluation of bowel tissues 10 days after induction of colitis showed marked reduction in inflammatory response and mucosal ulceration (1.34 versus 3.66 in tolerized versus nontolerized controls, respectively). In four of 20 recipients tolerized by enteral administration of colitis-extracted proteins, the colons were nearly normal by histological examination. In the rest of these rats, only minimal lymphocytic infiltration was detected. In contrast, a severe inflammatory reaction (grades 3 to 4) was observed in bowel specimens taken from nontolerized control rats. To evaluate the role of suppressor lymphocytes in oral tolerance induction, we tested whether lymphocytes harvested from tolerized rats can adoptively transfer the tolerance. Splenocytes were harvested from tolerized and nontolerized control rats and transplanted into naive irradiated animals, followed by rectal TNBS instillation. Rats were followed for clinical, macroscopic and microscopic manifestations of colitis. Splenocytes taken from tolerized donor rats fed with colitis-extracted proteins and transplanted into naive irradiated rats prevented TNBS-induced colitis in the recipients. The percentage of injured colonic area of total colonic wall and colon weight were significantly decreased in tolerized recipient rats. Similarly, the degree of colonic ulcerations, intestinal and peritoneal adhesions, wall thickness and degree of mucosal edema was markedly reduced in rats transplanted with splenocytes taken from tolerized donors and fed colitis-extracted proteins compared with rats transplanted with splenocytes taken from nontolerized controls. Tolerized recipient rats, transplanted with splenocytes taken from donors fed with colitis-extracted proteins, exhibited almost normal colonic mucosa. In contrast, when splenocytes were taken from nontolerized controls, severe disease developed upon rectal instillation of TNBS (46).

In tolerized rats fed colitis-extracted proteins, a Th2-type immune response was induced with secretion of high levels of TGF-β, along with downregulation of the Th1 immune response, manifested by a decrease in IFN-γ serum levels. In contrast, in nontolerized controls, a Th1-type immune response was observed with secretion of high levels of proinflammatory cytokines and downregulation of the secretion of anti-inflammatory cytokines.

Thus, oral tolerance toward colitis-extracted proteins downregulates the anticolon immune response, enabling effective treatment of experimental colitis. Tolerance is induced by suppressor lymphocytes, the transfer of which prevents colitis in naive animals. Tolerance is mediated by induction of Th2 type cytokines and inhibition of Th1 type cytokine secretion.

FUTURE DIRECTIONS IN ORAL TOLERANCE

Ongoing trials using oral tolerance aim to test the role of different subsets of intrahepatic lymphocytes in oral tolerance induction. The requirements for antigen presentation and cost stimulation as well as the cytokine milieu and roots of antigen processing are being studied. The role of subsets of lymphocytes (eg, gamma delta T cells) and the effects of oral

tolerance on antibody responses and on CTLs are being studied. The effect of oral tolerance on allergy, immunoglobulin E and other antibody response, and on various subgroups of cytotoxic T cells are also being investigated. Also explored are the effect of aging on tolerance induction, the use of transgenic plants for antigen presentation, the role of oral tolerance in the immune escape mechanism of tumours and the effect of immunomodulatory drugs on oral tolerance. The relationship between oral tolerance and food allergy or vaccinotherapy is being evaluated. Oral tolerance may develop as a new mode of treatment for various immune-mediated disorders. Immunosuppressive drugs are associated with short term and long term complications. In contrast, induction of specific tolerance toward disease-associated antigens may allow long term alleviation of the disease, leaving the general immunological defense of the recipient intact.

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