Institute of Experimental Morphology and Anthropology

Acta morphologica et anthropologica, 7
Sofia•2002

Oral Tolerance — A New Approach to the Treatment of Autoimmune Diseases

D. Nikolova, S. Makker**, E. Marinova*, B. Alexieva*, R. Alexandrova*, E. Bichkidzhieva**

**Institute of Experimental Morphology and Anthropology, Bulgarian Academy of Sciences, Sofia*
***University of California Davis, USA*

Oral administration of antigen can induce antigen-specific periferal immune tolerance known as oral tolerance (OT). It has been shown that oral autoantigen can suppress autoimmunity in different animal models of autoimmune diseases. Lately several studies have successfully demonstrated the effectiveness of OT suppressing desease activity in humans. Promissing results strongly suggest that OT induction is widely applicable to the treatment of human autoimmunity.

Key words: oral tolerance, autoantigen, autoimmune disease, T-lymphocytes, cytokines.

Oral tolerance (OT) is of unique immunologic importance because is the phenomen of antigen-specific hyporesponsiveness that results from oral administration of autoantigen [23]. Lately OT has been used successfully to treat autoimmune diseases in animal models and it is now being applied to the treatment of human diseases [7, 18, 25]. One of the first study to demonstrate that an orally administered autoantigen can suppress an autoimmune disease is in the model of arthritis [28]. It is established now that the suppression of collagen-induced arthritis by oral or nasal administration of collagen type II in experimental mice is provoked by induction of antigen-specific T-helper 2 (Th2) and transforming growth factor β(TGF-β) secreting regulatory cells. This is connected with suppression of local inflammation in joints and decreased T-helper 1 (Th1) type immune response in the periphery throughout the course of the illness [20]. In the model of experimental autoimmune encephalomyelitis (EAE) Lewis rat feeding with high doses of myelin basic protein (MBP) results in clonal anergy, whereas lower doses induce transferable cellular suppression [6, 26]. Mucosal administration of low doses of MBP peptide 68-86 or anti-inflammatory cytokine interleukin-10 (IL-10) effectively prevent EAE [27]. Lately it has been reported [18], that the use of copolimer 1(COP 1) induces OT which is effective in both the rat and mice model of EAE and it is may be more effective than MBP itself [18].

The authors demonstrate a dose-dependent suppression of EAE that can be adoptively transferred. The disease suppression is better seen in lower than in higher doses. The proposed mechanism of action of oral COP 1 is its bystander suppression [24]. These results raise the possibility that orally administered COP 1 may be an effective treatment for multiple sclerosis (MS) in human. It is shown that oral administration of retinal S-antigen, which induces experimental autoimmune uveitis suppresses the clinical signs of the disease [11]. Oral or nazal administration of large doses of antigen-acetylcholine receptor prevents or delays the onset of myastenia gravis (MG) in Lewis rats, which is an animal model of antibody-mediated disease [2, 3, 22]. The recombinant fragments of the acetylcholine receptor prevent also the induction of experimental autoimmune MG and suppresses ongoing MG in Lewis rats [14]. It was shown that the more native fragment of the antigen orally administered during the acute phase of disease leads to the exacerbation of MG. On the other hand, oral administration of the less native fragments of the antigen suppresses ongoing MG via the mechanism of T cell anergy. In the non-obese diabetic mouse model oral insulin has been shown to delay or prevent diabetes [29]. In Brown Norway rat model of interstitial nephritis, known as Th1 mediated disease, feeding of animals with renal tubular antigen (RTA) prior to the immunization whith RTA in complete Freund adjuvant (CFA) blocks dose-dependently the development of interstitial nephritis and renal insufficiency [13].

In the model of tubulointerstitial nephritis (TIN) in BALB/c mice oral administration of tubular basement membrane (TBM) antigen before and after induction of the disease leads to OT in autoimmune TIN [9]. It is shown that CD8 T cells are not involved in OT [21].

OT is an active immunologic process that is mediated by more than one mechanism. The nature of antigens, and the chemical structure of the fed tolerogen is important. Orally administered particulate antigens often induce an active immune response and the exacerbation of the disease, in contrast to the tolerance induced by the same antigens in soluble forms [14, 15]. This shows that the conformation of an orally induced antigen is a key factor in the induction of OT [17, 25].

Oral tolerance is antigen dose-dependent. Low doses of antigen administration favor the induction of active cellular regulation, whereas higher doses favor the induction of clonal anergy or deletion [8].

The microenvironment of the gut-associated lymphoid tissue (GALT) plays a central role in orally induced non-responsiveness by supporting the growth of regulatory T cells that maintain intestinal homeostasis in the face of constant antigenic challenge. GALT is a well developed immune network that consists of lymphoid nodules, epithelial villi, intraepithelial lymphocytes and other lymphocytes scattered throughout the lamina propria. Antigens may act directly at the level of the GALT or may exert their effects after absorbtion. Although dietary antigens are degradated by the time they reach the small intestine, studies in humans and rodents indicate that degradation is partial and some intact antigen is absorbed, especially when large doses are fed [5, 16]. Hence, OT can be explained with reference to antigen-presenting cell interactions with T cells in the GALT. The transfer non-responsiveness by peripheral T cells from antigen-fed animals suggest that these gut-derived regulatory cells also function in peripheral sites [10]. The higher doses of antigen induce clonal deletion or anergy of Th1 and Th2 cells which leads to OT. Recently it was found in the model of EAE in transgenic mice whose T cells carry T-cell receptor (TCR) specific for MBP that when the animals are fed with high doses of antigen T cells downregulate their TCR on the first day after feeding [4]. TCR returns to the cell surface by the third day after feeding. This fact dramatically affects the course of autoim-

mune response. Animals that were injected with the antigen on the first day after feeding were protected from the developing EAE, whereas those immunized on the third day were not. This study demonstrates that TCR-downregulation helps to define the mechanism of oral tolerance associated with high feeding dose.

The role of cytokines in the mechanism of OT is very important [1]. The lower doses of antigen induce Th2 and/or Th3 secreting regulatory cells and during active suppression of Th1 subset provoke an oral tolerance in Th1-dependent autoimmune diseases [14, 27].

But lately the traditionally sharp distinction between anergic and regulatory cells is beginning to diminish. It was established that the population of regulatory CD25+CD4+ cells express RNA for cytokines as TGF-β and IL-10. These cells are anergic and have suppressive properties that may act a non-specific fashion [19, 30].

The studies of OT in the field of experimental models of autoimmune nephritis are less (see above), but the positive results reported in Th1- mediated interstitial nephritis in rats, as in Th2-mediated tubulointerstitial nephritis in mice are promising.

Heyman nephritis (HN) is a rat experimental autoimmune model, prototype of human membraneous nephropathy, which frequently progress to end-stage kidney failure. HN is characterized with the formation of immune complexes (deposits) in the subepithelial region of glomerular basement membranes in kidney. The major target antigen for this disease is megalin (gp 600 kD). It is presented in the brush border of the epithelial cells in proximal renal tubules and in the glomerular epithelial cells. Our preliminary data have shown that the small N-terminal 60-kD fragment of megalin produces also a full-blown glomerulonephritis in rat [12]. Unlike megalin which is water- insoluble, 60-kD is prepared in soluble form, which is important for the induction of OT. Therefore this antigen (protein) can be used for the suppression of experimental glomerulonephritis via OT. Oral administration of this peptide prior or after the disease induction is expected to diminish the development of HN.

The effect of oral tolerance suppressing disease activity in different models of autoimmune diseases supports its application as a therapeutic option in autoimmune human disease. Currently, a multicenter, 1300 — patients, phase III clinical trial of an orally administered MBP analogue (glatiramer acetate) is in the progress. Now, an NIH-sponsored multicenter diabetes prevention trial is in the progress in which oral insulin is being given to children at risk for developing type 1 diabetes [23].

Although it is clear that oral antigen can suppress autoimmunity, much remains to be learned. Further areas of investigation include citokine milieu, form of the antigen and antigen presentation, costimulation requirements. As the molecular events associated with OT are better understood, the ability of its application to treat successfully human autoimmune diseases will be further enhanced.

References

1. Asano, M., M. Toda, N. Sakaguchi, S. Sakaguchi. Autoimmune disease as a consequence of developmental abnormality of T cell subpopulation. — J. Exp. Med., **12**, 1996, 387-396.
2. Baggi, F., F. Andreetta, E. Caspani et al. Oral administration of an immunodominant T-cell epitop downregulates Th1/Th2 cytokines and prevents experimental myasthenia gravis. — J. Clin. Invest., **104**, 1999, No 9, 1287-1295.
3. Barchan, D., O. Asher, S. Tzartos et al. Modulation of the anti-acetylcholine receptor response and experimental autoimmune myastenia gravis by recombinant fragments of the acethylcholine receptor. — Eur. J. Immunol., **28**, 1998, 616-624.

4. Benson, J. T-cell activation and receptor downmodulation precede deletion induced by mucosally administered antigen. — J. Clin. Invest., **106**, 2000, 1031-1038.
5. Bruce, M., A. Ferguson. Oral tolerance to ovalbumin in mice: studies of chemically modified and "biologically filtered" antigens. — Immunology, **4**, 1986, 627-630.
6. Chen, Y., V. Kuchroo, J. Inobe, H. Weiner. Regulatory T cell clones induced by oral tolerance: Suppression of autoimmune encephalomyelitis. — Science, **265**, 1994, 1237-1240.
7. Faria, A., H. Weiner. Oral tolerance: Mechanisms and Therapeutic Applications. — In: Advances in immunology, Academic Press, (Ed. Frank J. Dixon), 73, 1999, 153-264.
8. Friedman, A., H. Weiner. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. — Proc. Natl. Acad. Sci. USA, **91**, 1994, 6688-6692.
9. Marinova, E., D. Nikolova, D. Popova. Modulation of experimental autoimmune tubulointerstitial nephritis via oral treatment with bovine tubular basement membrane antigen. — In: 4th International conference on new trends in clinical and experimental immunosuppression. (Geneva, Switzerland, February, 17-20, 2000), Abstracts, p. 171.
10. Nagler-Anderson, C. Tolerance and immunity in the intestinal immune system. — Crit. Rev. Immunol., **20**, 2000, No 2, 103-120.
11. Nussenblatt, R., R. Caspi, R. Mahdi et al. Inhibition of S-antigen induced experimental autoimmune uveoretinitis by oral induction of tolerance with S-antigen. — J. Immunol. **144**, 1990, 1689-1695.
12. Oleinikov, A., B. Felitz, S. Makker. A small N-Terminal 60- kD fragment of gp600 (megalin), the major autoantigen of active Heyman nephritis, can induce a full-blown disease. — J. Am. Soc. Nephrol., **11**, 2000, 57-64
13. Pham, K., W. Smoyer, C. Archer et al. Oral feeding of renal tubular antigen abrogates interstitial nephritis and renal failure in Brown Norway rats. — Kidney Int., **52**, 1997, 725-732.
14. Sin-Hyeog, I., D. Barchan, S. Fuchs, M. Souroujon. Suppression of ongoing experimental myastenia by oral treatment with an acetylcholine receptor recombinant fragment. — J. Clin. Invest., **104**, 1999, No 12 , 1723-1730.
15. Sin-Hyeog, I., D. Barchan, M. Souroujon, M. Fuchs. Role of tolerogen conformation in induction of oral tolerance in experimental autoimmune myastenia gravis. — J. Immunology, **165**, 2000, 3599-3605.
16. Strobel, S., A. Mowat. Immune responses to dietary antigens: oral tolerance. — Immunol. Today, **4**, 1998, 173-181.
17. Strober, W., B. Kelsall, T. Marth. Oral Tolerance. — J. Clin. Immunol., **1**, 1998, 1-30.
18. Teitelbaum, D., R. Arnon, M. Sela. Immunomodulation of experimental autoimmune encephalomyelitis by oral administration of copolimer I. — Proc. Natl. Acad. Sci. USA, 7, 1999, 3842-3847.
19. Thornton, A., B. Shevach. Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. — J. Immunol., **1**, 2000, 183-190.
20. Trentham, D., R. Dynesius-Trentham, E. Orav et al. Effects of oral administration of collagen on rheumatoid arthritis. — Science, **261**, 1993, 1727-1730.
21. Vistica, B., N. Chanaud, N. Felix et al. CD8 T cells are not essential for the induction of "low-dose" oral tolerance. — Clin. Immunol. Immunopathol., **2**, 1996, 196-202.
22. Wang, Z., J. Qiao, H. Link. Suppression of experimental autoimmune myastenia gravis by oral administration of acetylcholine receptor. — J. Neuroimmunol., **44**, 1993, 209-214.
23. Weiner, H. Oral tolerance, an active immunologic process mediated by multiple mechanisms. — J. Clin. Invest., **106**, 2000, No 8, 935-937.
24. Wiener, H. Oral tolerance with copolymer 1 for the treatment of multiple sclerosis. — Proc. Natl. Acad. Sci. USA, **96**, 1999, 3333-3335.
25. Weiner, H., A. Friedman, A. Miller et al. Oral tolerance: Immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. — Annual. Rev. Immunol., **12**, 1994, 809-37.
26. Weiner, H. Oral tolerance: immune mechanisms and treatment of autoimmune diseases. — Immunol. Today, **18**, 1997, 335-343.
27. Xu, L., J. Yang, Y. Huang et al. Combined nasal administration of encephalitogenic myelin

basic protein peptide 68-86 and IL-10 suppressed incipient experimental allergic encephalomyelitis in Lewis rats. — Clin. Immunol., **96**, 2000, No 3, 205-211.
28. Zhang, J., C. Lee, O. Lider, H. Weiner. Suppression of adjuvant arthritis in Lewis rats by oral administration of type II collagen. — J. Immunol., **145**, 1990, 2489-2493.
29. Zhang, J., L. Davidson, G. Eisenbarth, H. Weiner. Suppression of diabetes in NOD mice by oral administration of porcine insulin. — Proc. Natl. Acad. Sci. USA, **88**, 1991, 10252-10256.
30. Zhang, X., L. Liu, T. Oida, H. Weiner. Oral tolerance induces regulatory CD25+CD4+ T cells in ovalbumin TCR transgenic mice. — FASEB J., **14**, 2000, A1198 (Abstract).