Zinc Deficiency Suppresses the Development of Oral Tolerance in Rats[1]

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ABSTRACT Oral tolerance is a specific immune unresponsiveness to food antigens to prevent hypersensitivity reactions. We investigated whether zinc deficiency affects oral tolerance. Rats were fed a control (C) or zinc-deficient (ZD) diet, or pair-fed (PF) to ZD rats for 28 d. Beginning on d 7, rats were administered ovalbumin (OVA) orally to induce tolerance, or PBS 3 times/wk, and were then immunized by OVA injection. The proliferation of mesenteric lymph node (MLN) and spleen lymphocytes after in vitro OVA stimulation and the delayed-type hypersensitivity were higher in OVA-fed ZD than in OVA-fed C rats and not different between OVA- and PBS-fed ZD rats, indicating a suppression of tolerance. Lymphocyte proliferation did not differ between PF and C rats. Expressions of cytokines involved in oral tolerance, i.e., interleukin (IL)-4, IL-10 and transforming growth factor-β, were higher in OVA- than in PBS-fed C rats, but not in ZD rats. Apoptosis was higher in OVA- than in PBS-fed C rats but not different between OVA- and PBS-fed ZD rats. Inflammation and ulcerations that were not present in ZD rats on d 7 (ZD_7) developed in OVA- or PBS-fed ZD rats. Compared with ZD_7 rats, tumor necrosis factor-α and cytokine-induced neutrophil chemoattractant were higher in OVA- and PBS-fed ZD rats, whereas interferon-γ increased only in OVA-fed ZD rats. In conclusion, zinc deficiency suppresses oral tolerance through dysregulation of cytokine expression and lack of antigen-specific clonal deletion. We suggest that abrogation of tolerance may lead to development of mucosal inflammation and damage. J. Nutr. 133: 191–198, 2003.

KEY WORDS: • *zinc deficiency* • *oral tolerance* • *intestine* • *cytokines* • *rats*

The intestinal mucosa is an enormous surface that is continuously exposed to a myriad of intraluminal antigens. The immune system of the gut has to be able to protect the mucosa against pathogens but also must avoid hypersensitivity reactions to food proteins, normal bacterial flora and other environmental macromolecules. Oral tolerance is a specific suppression of cellular and humoral cell-mediated immune responses to orally administered antigen upon subsequent immunization with the same antigen to prevent immune reactions to dietary antigens (1–6). There is a large body of evidence that adverse reactions to foods have an immunological basis and may represent a suppression of tolerance to components of gut flora and food (2,7). Breakdown of oral tolerance may lead to the development of mucosal immunopathology directed against environmental antigens or autoantigens and thus to autoimmune diseases. Indeed, oral tolerance has been employed successfully for treatment of human autoimmune diseases (1) and to suppress experimental autoimmune myasthenia gravis (8), uveitis (9), rheumatoid arthritis (10), autoimmune encephalomyelitis (11) and colitis (12).

Tolerance may occur by a number of mechanisms depending on the dose and nature of the antigen orally administered. The induction of clonal deletion of antigen-specific T lymphocytes and anergy of T cells have been demonstrated to occur after high doses of oral antigen (13,14). Feeding a low antigen dose is generally associated with active suppression (1,15). This is generated by food antigen uptake and processing in Peyer's patches and villous epithelium, which induce the development of T-helper (Th)[4]2 cells secreting immunosuppressive cytokines such as interleukin (IL)-4 and IL-10, as well the development of Th3 lymphocytes producing transforming growth factor (TGF)-β (16). These cytokines can suppress immune reactions and antagonize the expression of Th1 proinflammatory cytokines. The Th2 cells can emigrate to peripheral sites where they prevent continuing activation of pathogenic Th1 cells. Although it is generally believed that active suppression and apoptosis of T-cells act separately in induction of tolerance, there is also evidence that both these mechanisms may be present at the same time in animals in which tolerance was induced (17,18).

In addition to the dose or nature of antigen, it has been suggested that altered immunological status, increased inflammation, dysfunction or damage in the epithelial barrier and

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[4] Abbreviations used: C, control; CFA, complete Freund's adjuvant; CINC, cytokine-induced neutrophil chemoattractant; ConA, concanavalin A; DTH, delayed-type hypersensitivity; FCS, fetal calf serum; GAPDH, glyceraldheyde-3-phosphate-dehydrogenase; IFN, interferon; IL, interleukin; MLN, mesenteric lymph nodes; MPO, myeloperoxidase; OVA, ovalbumin; PF, pair-fed; RT-PCR, reverse transcription-polymerase chain reaction; TGF, transforming growth factor; Th, T-helper; TNF, tumor necrosis factor; ZD, zinc deficient.

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