Available online at www.sciencedirect.com
SCIENCE DIRECT
Immunobiology 211 (2006) 157–166
Immunobiology
www.elsevier.de/imbio

Long-term anergy in orally tolerized mice is linked to decreased B7.2 expression on B cells

Eliana A. Futata, Cyro A. de Brito, Jefferson Russo Victor, Ana Elisa Fusaro, Célia R. Oliveira, Milton Maciel Jr, Alberto José da Silva Duarte, Maria Notomi Sato*

Laboratório de Dermatologia e Imunodeficiências, Faculdade de Medicina da Universidade de São Paulo, Instituto de Medicina Tropical – Prédio II, Av Dr Enéas de Carvalho Aguiar, 500, 3° Andar, 05403-000- São Paulo, Brazil

Received 13 June 2005; accepted 23 August 2005

Abstract

Durable antigen (Ag)-specific T- and B-cell anergy induced by oral tolerance is an attractive strategy for immunotherapy of allergic diseases. Here, we address the lasting effect of oral tolerance induction in naïve or primed mice to ovalbumin (OVA) on antibody production. Single feeding with OVA prior to immunization or double feeding, before and after Ag priming, in A/Sn mice, induced a long-lasting suppression of IgE, IgG1 and IgG2a responses up to 8 months after immunization. In contrast, primed-fed mice had transient IgE inhibition. Naive and double-treated mice showed marked Ag-specific unresponsiveness and scarce cytokines production. Inhibition of IL-2 and IFN-γ secretion in naïve-fed mice were restored in the presence of anti-CD28 mAb plus Ag stimulation. The durable inhibition of Ab production in OVA-fed mice was related to the persistent decrease of B7.2 expression on B cells. Ag feeding in naive and primed status may be a prophylactic measure to avoid later Ag sensitization.

Keywords: Anergy; Costimulatory molecules; Cytokines; IgE; Oral tolerance

Introduction

Oral tolerance refers to systemic antigen (Ag) hyporesponsiveness that occurs after oral Ag administration followed by a challenge with the same Ag (Mowat, 1987). Experimental models have shown that, depending on the Ag dose ingested, tolerance can be mediated through suppression, clonal deletion or anergy (Melamed et al., 1996; Van Houten and Blake, 1996; Weiner, 1997). Low and multiple Ag doses favor immunosuppression (Kjaer and Frokiaer, 2002; Mucida et al., 2005) while high doses result in clonal deletion and T-cell anergy (Faria et al., 2003; Wakabayashi et al., 1999).

Several types of oral tolerance regimens, usually in naive animals, have been addressed to obtain Ag specific unresponsiveness at T- and B-cell level. Although prevention of sensitization through Ag feeding has been demonstrated (Weiner, 1997), it seems difficult to revert existing immune responses through oral tolerance induction (Torseth and Gregerson, 1998; Leishman et al., 1998). Attempts with different regimens to induce oral tolerance are aimed at prophylaxis to Ag sensitization and/or therapeutic action on existing immune responses.

*Corresponding author. Tel.: + 55 11 3066 7499; fax: + 55 11 3081 7190.
E-mail address: marisato@usp.br (M.N. Sato).

0171-2985/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.imbio.2005.08.006

Maintenance of T-cell tolerance is an ongoing process that is necessary for prevention of autoimmunity and allergic diseases. Anergy is characterized by failure of cellular proliferation and IL-2 secretion upon Ag receptor engagement (Van Houten and Blake, 1996; Omata et al., 2005) which could be related to the lack of costimulatory signal. Costimulatory molecules, including B7.1 (CD80) and B7.2 (CD86) – expressed on Ag-presenting cells – are specific for both the stimulatory receptor CD28 and the inhibitory receptor Cytotoxic T-Lymphocyte Associated Antigen (CTLA-4, CD152) (Fowler and Powrie, 2002). In vitro stimulation with anti-CD28 mAb can provide a costimulatory signal to naïve T CD4+ cells and T-cell clones blocking the induction of anergy (Chung et al., 2003). On the other hand, engagement of CTLA-4 limits the degree of T-cell activation (Chen et al., 2002). This inhibitory signaling seems to be active in oral tolerance since complete abrogation of T-cell tolerance induced by high dose Ag could be obtained by selective blockage of CTLA-4 (Samoilova et al., 1998).

The crucial role of B7 molecules on antibody production could be seen in mice deficient for B7.1 and B7.2 that failed to generate Ag-specific IgG1 and IgG2a responses and lacked germinal centers (Borriello et al., 1997). In another instance, B7 role was evidenced by lack of oral tolerance induction in CD40−/− mice (Kweon et al., 1999). Indeed CD40L-CD40 interaction up-regulates B7.1 and B7.2 expression (Sharp and Freeman, 2002).

Furthermore, an enhanced B7.2 expression on B cells has been observed in a murine model for atopic dermatitis to house dust mite (Santa et al., 2003). However, it is not known whether maintenance of anergy induced by high Ag feeding could be related to a long permanence of altered B7 expression since circulating Ag may disappear during this time.

In the present work, we sought to investigate the durability of the effects triggered by several regimens in oral tolerance to ovalbumin (OVA), in naive or Ag primed mice, on antibody production and the costimulatory B7 molecules expressed on B cells. Moreover, the involvement of CTLA-4 expression in the anergic process induced by oral tolerance was further ascertained.

Materials and methods

Animals

Male A/Sn mice 8–10 weeks old were provided by the animal facility of the School of Medicine – University of São Paulo. Wistar Furth rats ($RT1^{u,u}$) of both sexes, 3–4 months old, bred in our own animal facility were used for passive cutaneous anaphylaxis (PCA) reactions.

Immunization protocol

Groups of mice were immunized intraperitoneally with 50 μg of OVA (grade V; Sigma, St. Louis, MO) in 0.1 mg of aluminium hydroxide [$Al(OH)_3$, alum]. Mice were boosted intraperitoneally on the 10th and 60th or 120th or 240th days after immunization with 50 μg of OVA in saline solution. Groups of mice were bled 7 days after each boost and serum samples were frozen at −70 °C until use.

Oral antigen administration protocol

Mice were intragastric (ig) administered with saline (ig-saline) or with 25 mg of OVA in 0.5 ml of saline by gavage under light ether anaesthesia within 3 (ig−3) or 7 (ig−7) days before priming (dbp) or 3 (ig+3) or 7 (ig+7) days after priming (dap). Groups were treated three dbp and three dap (ig−/+3) or seven dbp and seven dap (ig−/+7) or seven dbp and three dap (ig−7/+3) with a total of 50 mg of OVA. Some groups were fed with OVA 21 (ig−21) or 14 (ig−14) days before priming. Other groups were fed with 25 mg of OVA three dap and, 42 days after the first feeding, were re-treated with 100 mg of Ag (ig +3/+45). All groups were boosted on the 10th day after immunization.

Passive cutaneous anaphylaxis (PCA)

IgE antibodies were estimated by PCA reaction as described by Mota and Wong (1969). Briefly, 0.1 ml of serum dilutions from each mouse was inoculated intradermally on the shaved back of the rats. After 18 h, the rats received 0.5 mg of OVA in 1.0 ml 0.5% Evans Blue by tail vein injection; 1 h later, the rats were killed, and the reciprocal of the highest serum dilution that produced a spot larger than 5 mm in diameter was taken as the PCA titer.

Determination of antibody levels

An enzyme-linked immunosorbent assay (ELISA) was performed to detect IgG isotypes as previously described (Sato et al., 2002). Briefly, 96-well microplates (Costar, Cambridge, MA) were coated with 5 μg/ml of OVA in 0.1 M carbonate–bicarbonate buffer, pH = 9.5. The wells were blocked with PBS-0.5% bovine serum albumin (BSA, Sigma, St. Louis, MO) before incubation of the serum samples for 1 h at 37 °C and overnight at 4 °C. Detection was performed with biotinylated anti-γ1 or anti-γ2a mouse chain antibodies (1:3000, Southern Biotechnology Ass, Birmingham, AL) and streptavidin-peroxidase (1:200,000, Sigma, St. Louis, MO) and TMB (3,3′, 5,5′-tetramethyl-benzidine, Sigma). The reaction was stopped with sulphuric acid and read at 450 nm in