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A diet enriched with cocoa prevents IgE synthesis in a rat allergy model

Mar Abril-Gil[a], Malén Massot-Cladera[a], Francisco J. Pérez-Cano[a,b], Cristina Castellote[a,b], Àngels Franch[a,b], Margarida Castell[a,b,*]

[a] Departament de Fisiologia, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII s/n, E-08028 Barcelona, Spain
[b] Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), E-08028 Barcelona, Spain

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ABSTRACT

Previous studies in young rats reported the impact of cocoa intake on healthy immune status and allow suggesting it may have a role in the prevention of some immune-mediated diseases. The aim of this study was to ascertain the effect of a cocoa diet in a model of allergy in young rats. Three-week-old Brown Norway rats were immunized by i.p. injection of ovalbumin (OVA) with alum as adjuvant and *Bordetella pertussis* toxin. During the next 4 weeks rats received either a cocoa diet (containing 0.2% polyphenols, w/w) or a standard diet. Animals fed a standard diet showed high concentrations of anti-OVA IgG1, IgG2a, IgG2b and high anti-OVA IgE titres, which is the antibody involved in allergic response. In contrast, animals fed a cocoa diet showed significantly lower concentrations of anti-OVA IgG1 and IgG2a antibodies. Interestingly, the cocoa diet prevented anti-OVA IgE synthesis and decreased total serum IgE concentration. Analysis of cytokine production in lymph node cells at the end of the study revealed that, in this compartment, the cocoa diet decreased the tumor necrosis factor (TNF)-α and the interleukin (IL)-10 secretion but not IL-4 production. In conclusion, a cocoa-enriched diet in young rats produces an immunomodulatory effect that prevents anti-allergen IgE synthesis, suggesting a potential role for cocoa flavonoids in the prevention or treatment of allergic diseases.

1. Introduction

Allergy is a common form of hypersensitivity with an incidence that has increased dramatically in the developed world during the last 50 years and, at present, it affects more than 25% of the population in these countries [1]. Atopic individuals show a dysregulated immune response to non-pathogenic proteins, called allergens, present either in the environment (*i.e.*, dust, pollen, etc.) or in food (*i.e.*, eggs, milk, nuts, etc.). This response starts, like any acquired immune response, with antigenic sensitization. Allergen is taken by dendritic cells and presented to specific Th cells by means of major histocompatibility complex (MHC) class II and co-stimulatory molecules (CD80, CD86, etc.) thus performing the immunological synapse [2]. Activated allergen-specific Th cells, which usually polarize in the Th1 or Th2 effector populations, differentiate and expand into a Th2 subpopulation. Activated Th2 cells produce cytokines such as interleukin (IL)-4, IL-5, IL-10, and IL-13 which are important in switching antibody production from B cells to predominantly IgE production against the allergen, as well as stimulating mast cells and eosinophils. Specific IgE binds then to IgE receptors (FcεRI) in mast cells; when allergen crosslinks to mast cell-bound IgE, a large number of preformed and newly synthesized mediators and proteases are released and they are responsible for allergic manifestations [3]. Symptoms of allergy affect the skin, respiratory and gastrointestinal systems or even the nervous and cardiovascular systems. The most severe form of allergy is the anaphylaxis, which involves two or more organic systems, sometimes producing an anaphylactic shock with hypotension that can result in death.

At present, the majority of pharmacological agents available for allergies (corticosteroids and antihistamines) are aimed at treating different allergic symptoms. Although they are highly effective, numerous factors, such as the dose and the route of administration, account for the efficacy of these treatments, and adverse effects are sometimes associated with a chronic use of these drugs [4].

Abbreviations: AU, arbitrary units; BSA, albumin from bovine serum; CBA, cytometric bead array; FBS, fetal bovine serum; IFN, interferon; IL, interleukin; MLN, mesenteric lymph nodes; OPD, o-phenylenediamine; OVA, ovalbumin; PBS-Tw, phosphate-buffered saline containing 0.05% Tween 20; RT, room temperature; tBp, *Bordetella pertussis* toxin; TNF, tumor necrosis factor.

* Corresponding author at: Departament de Fisiologia, Facultat de Farmàcia, Universitat de Barcelona, Av Joan XXIII s/n, Edifici B, 3ª planta, E-08028 Barcelona, Spain. Tel.: +34 93 402 45 05; fax: +34 93 403 59 01.
E-mail addresses: mariadelmar.abril@ub.edu (M. Abril-Gil), malen.massot@ub.edu (M. Massot-Cladera), franciscoperez@ub.edu (F.J. Pérez-Cano), cristinacastellote@ub.edu (C. Castellote), angelsfranch@ub.edu (À. Franch), margaridacastell@ub.edu (M. Castell).

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Therefore, research into new anti-allergic therapy must be performed in order to make it effective from the prevention of allergic sensitization until the alleviation of allergy symptoms. Recent studies have highlighted different nutritional interventions that can impact on allergic diseases [5]. Dietary polyphenols have been found among these emerging nutraceuticals [6].

Flavonoids are products of the secondary metabolism of plants that are regularly ingested in small quantities in many edible plants. Chemically, they have a polyphenolic structure showing antioxidant activities. These properties have aroused an increasing interest in assessing their possible beneficial role in the prevention of various diseases, as evidenced by the large number of studies focused on the effect of flavonoids on health over the last decade [7,8]. Considering the role of flavonoids in the immune system, most studies are performed *in vitro*, and those reports focusing on acquired immune response suggest that flavonoids produce an inhibitory effect on lymphocyte activation [9–11]. These studies show that flavonoids can affect both early and late phases of the immune response, including Th1 and Th2 effector responses, although a skewed effect of flavonoids favouring or inhibiting Th1 or Th2 responses has not been clearly established. Most preclinical studies on flavonoids in the acquired immune response use an antigen sensitization model followed by a challenge through different routes, searching to provoke a harmful immune response. Different types of flavonoids show the potential to suppress these damaging responses [12–14].

Cocoa is a rich source of polyphenols, particularly flavonoids such as (−)-epicatechin and (+)-catechin as well as procyanidins, the polymers derived from these monomers [15]. Previous studies demonstrated that a cocoa-enriched diet in rats was able to modify the composition and functionality of several lymphoid tissues [16–19], decreasing serum IgG, IgM and IgA concentrations [16]. In addition, a cocoa diet in rats immunized with ovalbumin (OVA) attenuated anti-OVA IgG1 (the main isotype associated with the Th2 immune response in rats), IgG2a, IgG2c and IgM concentrations but led to higher amounts of anti-OVA IgG2b (the isotype linked to the Th1 response) [18]. Similarly, a cocoa diet was able to attenuate the specific antibody response in a rat model of chronic inflammation [19].

In light of this background which demonstrates the modulatory effect of cocoa on the immune system, the aim of this study was to ascertain whether a cocoa-enriched diet could also attenuate the synthesis of IgE in a rat allergy model.

2. Materials and methods

2.1. Chemicals

The Natural Forastero cocoa (Nutrexpa, Barcelona, Spain) used in this study presented a total polyphenol content of about 22 mg/g with 2.2 mg/g (−)-epicatechin, 0.74 mg/g (+)-catechin and 1.7 mg/g procyanidin B2. Ovalbumin (OVA, grade V), albumin from bovine serum (BSA), gelatine, peroxidase-conjugated extravidin, o-phenylenediamine (OPD), and toxin from *Bordetella pertussis* (tBP) came from Sigma–Aldrich (Madrid, Spain). Imject® alum was obtained from Pierce (Rockford, IL, USA). Purified rat IgE, anti-rat IgE monoclonal antibody and biotin-conjugated anti-rat IgG1, IgG2a, IgG2b and IgE monoclonal antibodies, and rat standard, capture bead and detection reagent for interleukin (IL)-2, IL-4, IL-10, interferon (IFN)-γ and tumor necrosis factor (TNF)-α were purchased from BD Biosciences (Heidelberg, Germany). The biotin protein labelling kit used was from Roche Diagnostics (Mannheim, Germany). RPMI 1640 medium, foetal bovine serum (FBS), L-glutamine, streptomicin and penicillin were obtained from PAA (Pasching, Austria). β-Mercaptoethanol was provided by Merck (Darmstadt, Germany).

2.2. Diets

The AIN-93G formulation (Harlan) was used as the standard diet. Previous studies have demonstrated that this diet does not contain detectable amounts of polyphenols [20]. A 10% cocoa diet was produced with a modification of the AIN-93G formula, consisting of subtracting the amount of carbohydrates, proteins, lipids and fibre provided by the corresponding percentage of cocoa as previously described [16]. Consequently, the resulting chow was isoenergetic with the standard diet.

2.3. Experimental design and induction of rat allergy model

Brown Norway rats were purchased from Janvier (France) and maintained in the animal facilities of the Faculty of Pharmacy under conditions of controlled temperature and humidity in a 12:12 light:dark cycle. At weaning (day 21 of life) the rats were housed in polycarbonate cages (three to four per cage) and were randomized into two dietary groups: the cocoa group, formed by animals fed chow containing 10% cocoa starting at weaning and continuing until the end of the study (4 weeks later), and the reference group, formed by animals fed standard chow.

At weaning, the rat allergy model was induced using OVA as allergen and tBp to promote IgE synthesis [21]. Each rat was injected i.p. with 0.5 mL of an emulsion containing 0.5 mg of OVA together with 50 ng of tBp emulsified with Imject® (3:1 OVA:Alum). Blood samples were collected at the beginning of the study (before induction) and at 3 and 4 weeks after allergy induction. At week fourth, mesenteric lymph nodes (MLN) were obtained. Lymphocytes from MLN were immediately isolated in sterile conditions by passing the tissue through a mesh (100 μm, BD Biosciences). The cell suspension was left on ice for 10 min to remove tissue debris by sedimentation. Later, cells were centrifuged ($600 \times g$, 5 min, 4 °C) and resuspended in RPMI 1640 medium supplemented with 10% FBS, 100 IU/mL streptomycin–penicillin, 2 mM L-glutamine, and 0.05 mM 2-mercaptoethanol. MLN cells were cultured at 5×10^6 cells in 1 mL with OVA (50 μg/mL) for 72 h (37 °C, 5% CO_2). Supernatants were then collected to assess cytokine concentrations.

Experimental procedures were reviewed and approved by the Ethical Committee for Animal Experimentation of the University of Barcelona.

2.4. Quantification of anti-OVA IgG1, IgGa and IgG2b antibodies

Serum anti-OVA IgG1, IgG2a and IgG2b concentrations were quantified by ELISA as previously described [18]. In brief, 96-well polystyrene plates (Nunc MaxiSorp, Wiesbaden, Germany) were coated with OVA (10 μg/mL in PBS) and, after blocking with 0.5% gelatine, appropriate diluted samples (ranging from 1/50 to 1/500,000) were added. After washing, biotin-conjugated anti-rat IgG1, IgG2a or IgG2b antibodies and subsequently, peroxidase-conjugated extravidin were added. An OPD-H_2O_2 solution was used for the detection of bound peroxidase. OD was measured on a microtiter plate photometer (Labsystems Multiskan, Helsinki, Finland) at 492 nm. Data were interpolated by means of Ascent v.2.6 software (Thermo Fisher Scientific, S.I.U., Barcelona, Spain). The relative concentration of each anti-OVA isotype was calculated by comparison with a pool of OVA-immunized rat sera to which arbitrary units (AU) were assigned according to the dilution of the serum samples used for each isotype determination (100,000 AU/mL for IgG1 and IgG2a; 10,000 AU/mL for IgG2b).