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Immunogenicity and Modulatory Effect of the Lectins from *Artocarpus heterophyllus* (Jackfruit) Seeds, Artocarpin and Jacalin

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SUMMARY. The immune response of mice subcutaneously immunized with two lectins from *Artocarpus heterophyllus* seeds, artocarpin and jacalin, and their possible modulatory effect on antibody synthesis of mice immunized with an unrelated antigen, were studied. Both lectins induced specific synthesis of antibodies, irrespective of their immunizing dose. Concerning the modulatory effect on the synthesis of anti-ovalbumin total immunoglobulins, artocarpin stimulated the synthesis of anti-ovalbumin antibodies irrespective of its dose and jacalin had a tendency to stimulate such antibody synthesis according to its dose. Discrimination of anti-ovalbumin synthesis of IgG1 and IgE showed that the artocarpin modulated IgG1 whilst jacalin modulated IgE.

RESUMEN. "Inmunogenicidad y efecto modulador de dos lectinas de semillas de *Artocarpus heterophyllus*, artocarpina y jacalina". Se estudió la respuesta inmune de ratones inmunizados subcutáneamiente con dos lectinas de semillas de *Artocarpus heterophyllus*, artocarpina y jacalina, y sus posibles efectos moduladores en la síntesis de anticuerpos de ratones inmunizados con un antígeno no relacionado estructuralmente. Las dos lectinas inducen la síntesis específica de anticuerpos, independientemente de sus dosis inmunizantes. Con respecto al efecto modulador sobre la síntesis de inmunoglobulinas totales antiovoalbúmina, la artocarpina estimuló la síntesis de anticuerpos antiovoalbúmina independientemente de su dosis y la jacalina tuvo tendencia a estimular la síntesis del anticuerpo según su dosis. La discriminación de las síntesis de IgG1 y IgE anti-ovoalbumina demostró que la artocarpina modula la IgG1, en tanto que la jacalina modula la IgE.

INTRODUCTION

Jacalin and, more recently, artocarpin, are the main lectins from *Artocarpus heterophyllus*, which are used as tools in immunological studies [1,2]. Jacalin is a D-galactose-specific lectin, known for its reactivity towards human IgA and IgD [3,4]. In addition, it has been described to interact with CD4 molecules [5] and recently it was shown that jacalin induces interleukin-6 secretion, using cells from monocyte/macrophage lineage that express the CD4 molecule [6]. Artocarpin, a term first used for the α-D-galactosyl lectin from *Artocarpus lakoocha* seeds [7], has also recently been applied to another lectin found in *Artocarpus integrifolia* seeds with carbohydrate specificity towards D-mannose. This lectin was characterized as a T cell-dependent B cell polyclonal activator [2].

Despite their use in immunological studies, jacalin and artocarpin have rarely been used for *in vivo* studies related to antibody synthesis. In the present work we have evaluated the immune response induced by jacalin and artocarpin and compared the immunomodulatory effects exerted by different doses of these lectins on the antibody synthesis induced by a non related antigen, ovalbumin (OVA). Our data showed a modulatory effect of artocarpin and jacalin on anti-ovalbumin total immunoglobulin synthesis and a different modulation by these lectins on anti-Ova IgG1 and IgE isotypes.

KEY WORDS: Artocarpin, IgE, IgG1, Immunemodulation, Jacalin, Lectin.
PALABRAS CLAVE: Artocarpina, IgE, IgG1, Inmunomodulación, Jacalina, Lectina.

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MATERIALS AND METHODS

Mice

Swiss mice were genetically controlled by the Centro de Bioterismo of UNICAMP, Campinas, SP, Brazil and maintained at the Biotério Central of the Universidade Federal do Ceará.

Lectins

The lectins, artocarpin and jacalin, were purified from seeds of jackfruit, collected in Riacho da Guia (State of Bahia, Brazil) according to Mourão *et al.* [8].

Immunization

Groups of ten mice were immunized by subcutaneous injection with 10 µg OVA, 10 µg OVA plus 5 (LD) and 50 (HD) µg either artocarpin (F1) or jacalin (F2). All mice were boosted on days 21 and 35 after primary immunization. The mice were bled from the orbital plexus on days 7, 14, 21, 28, 35, and 42 after the first injection and the sera obtained were stored at -20 °C until use.

Antibody assays

The antibody levels to OVA, artocarpin and jacalin antigens were assayed by ELISA (Enzyme Linked Immunosorbent Assay) using pooled sera from each group of animals. For this assay, microtiter plates (Falcon, Lincoln Park, NJ) were coated with appropriate antigen (10 µg/ml) in a volume of 50 µl/well and incubated overnight at 4 °C. After blocking with 5% non-fat milk in PBS, pH 7.2, for 2 h at room temperature, 50 µl of the appropriate sera diluted in PBS were added and incubated for 1 h at room temperature. The plates were then washed five times with PBS-Tween-20 (0.05%) and treated with peroxidase-conjugated rabbit anti-mouse immunoglobulins (50 µl/well 1:1000 Dako No. P 260. DK-2600 Glostrup Denmark) for 2 h at room temperature. The plates were subsequently washed five times with PBS-Tween. The reaction was developed by the addition of H_2O_2 and orthophenylenediamine (OPD) followed by incubation for 20 min at 37 °C. The reaction was stopped by the addition of 20 µl of 2.5 N H_2SO_4 and the intensity of the resulting color then read at 492 nm using a Titertek Multiskan reader. The results are reported as ELISA, which is the mean of the sums of absorbance values read between 1/100 and 1/12,800 serum [9].

IgG1 and IgE antibodies were measured in terms of the ability of antisera to induce PCA (Passive Cutaneous Anaphylaxys). IgE antibodies were measured in rats [10]. The interval between skin sensitization and challenge was 18 h; for challenge an intravenous injection of 1 ml of a solution containing 0.5% Evans blue in saline and 2.0 mg OVA was used. IgG1 antibodies were measured in terms of the ability of the same antisera to induce PCA in mice [11] after an interval of 2 h between skin sensitization and challenge by intravenous injection of 0.25 ml of a solution containing 0.5% Evans blue in saline and 0.5 mg OVA.

PCA titers were expressed as log 2 of the inverse of the highest dilution giving a positive reaction. Each serum sample was assayed in at least 2 rats for IgE or in 4 mice for IgG1 antibodies. Controls were performed in every rat or mouse with a known standard serum: the reaction given by the sera tested was weighted in relation to the titer of the standard serum [12]. Controls without sera or antigen in the challenging solutions gave negative results.

Statistical analysis

Statistical analysis was performed using a two-way analysis of variance (ANOVA) either in a classical or in a totally additive model. Differences between group means were evaluated by the Tukey range test procedure with values of $P \leq 0.05$ or $P \leq 0.01$, respectively, being considered significant [13].

RESULTS

Immunogenicity of artocarpin and jacalin

Sera from animals immunized with OVA associated with 5 (LD) and 50 (HD) µg of artocarpin and jacalin were used to determine the specific immunoglobulins against both lectins by ELISA. Specific antibodies against artocarpin and jacalin were not detected on primary immune response (data not shown). Contrarily, antibodies against artocarpin (F1) and jacalin (F2) were detected after a booster (on day 28) and were maintained thereafter (Figure 1). However, the levels of specific antibodies induced by jacalin were higher than those induced by artocarpin (Figure 1). Furthermore, the immune response induced by jacalin and artocarpin were dose-independent.

Modulatory effect of artocarpin and jacalin on the synthesis of anti-OVA total immunoglobulins

Mice inoculated with OVA plus artocarpin (F1) or jacalin (F2) 5 or 50 µg, developed an an-

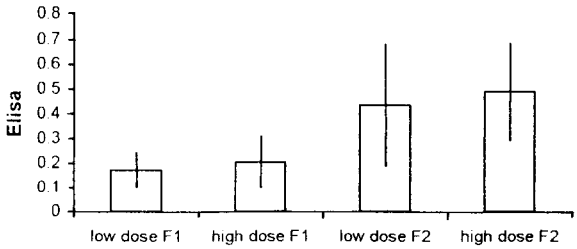

Figure 1. Anti-artocarpin and anti-jacalin specific antibody production on secondary immune response (28 days).

Swiss mice (10/group) were immunized with 5 (low dose) and 50 µg (high dose) either artocarpin (F1) or jacalin (F2) and on days 21 and 35 after primary immunization, booster injections were given in the same conditions. Seven days after the first booster (day 28) mice were bled and the levels of specific antibodies to artocarpin and jacalin were determined. The results are expressed as the mean ± SEM of the sums of absorbance values read between 1/100 and 1/12,800 serum.

tibody response to OVA higher than that of the group immunized with OVA alone (Figure 2). Artocarpin exhibited a tendency to enhance anti-OVA antibody synthesis independently of its dose whereas jacalin had a tendency to enhance anti-OVA antibody synthesis according to its dose.

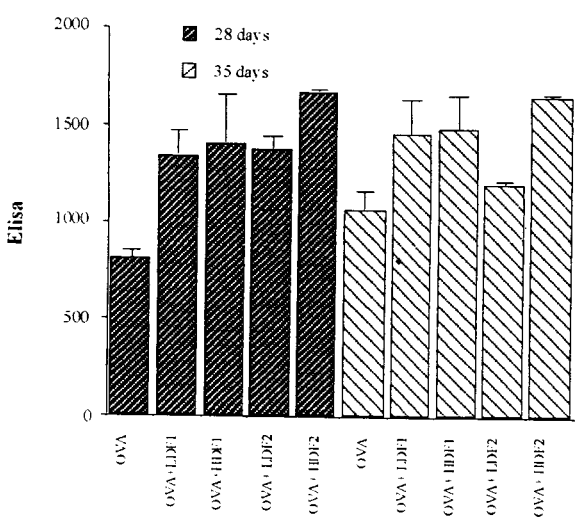

Figure 2. Modulatory effect of artocarpin and jacalin on the antibody production to OVA.

Swiss mice (10/group) were immunized with OVA in the absence or presence of 5 (LD) and 50 (HD) µg either artocarpin (F1) or jacalin (F2) and on days 21 and 35 after primary immunization, booster injections were given in the same conditions. One week post-booster, the mice were bled and their sera assayed by ELISA for the presence of Ig to OVA. The results are expressed as the mean ± SEM of the sums of absorbance values read between 1/100 and 1/12,800 serum.

Regulatory effect of artocarpin and jacalin on anti-OVA isotypes, IgG1 and IgE

In order to characterize the immunomodulatory effect of artocarpin (F1) on anti-OVA isotypes IgG1 and IgE, sera from mice, immunized as previously described, were analyzed for IgG1 and IgE by PCA and compared with the effect of jacalin (F2) (Figures 3 and 4). Since the statistical analysis revealed an interaction between the factor time and the treatments of 5 and 50 µg of artocarpin and jacalin, the results were punctually time-controlled. The data showed that 5 µg artocarpin had a significant up-regula-

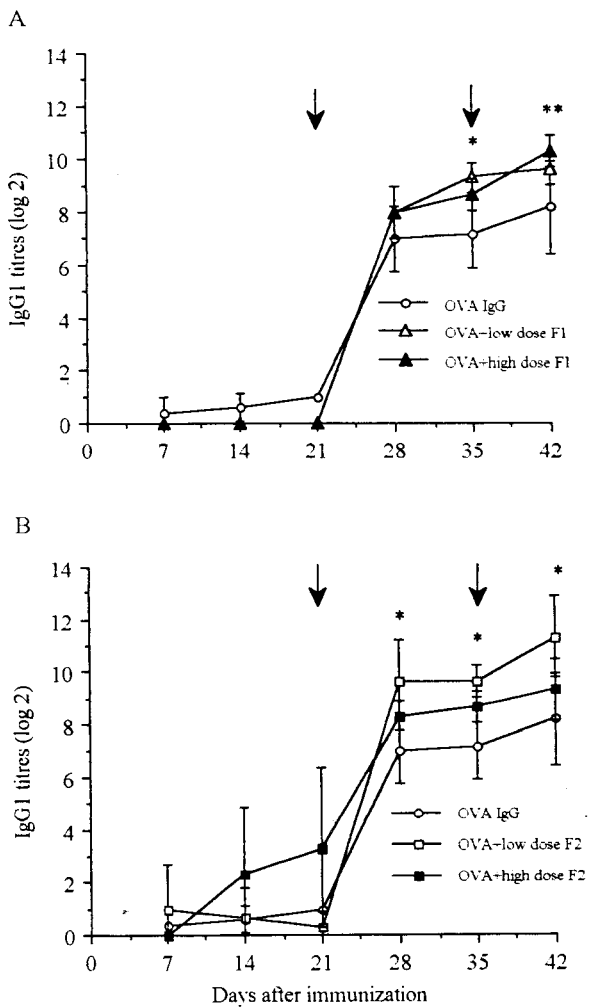

Figure 3. Kinetics of anti-OVA IgG1 antibody response modulated by artocarpin (F1) and jacalin (F2).

Swiss mice (10/group) were immunized with OVA in the absence or presence of 5 (low dose) and 50 µg (high dose) either F1 (A) or F2 (B) and on days 21 and 35 after primary immunization, booster injections were given in the same conditions (indicated by the arrows). The effect of 5 µg F1 (artocarpin) was significant at days 35 and 42 and the effect of 50 µg F1 (artocarpin) was significant at day 42. In all cases $P \leq 0.05$. The effect of 5 µg F2 (jacalin) was significant at days 28, 35 and 42 with $P \leq 0.05$.

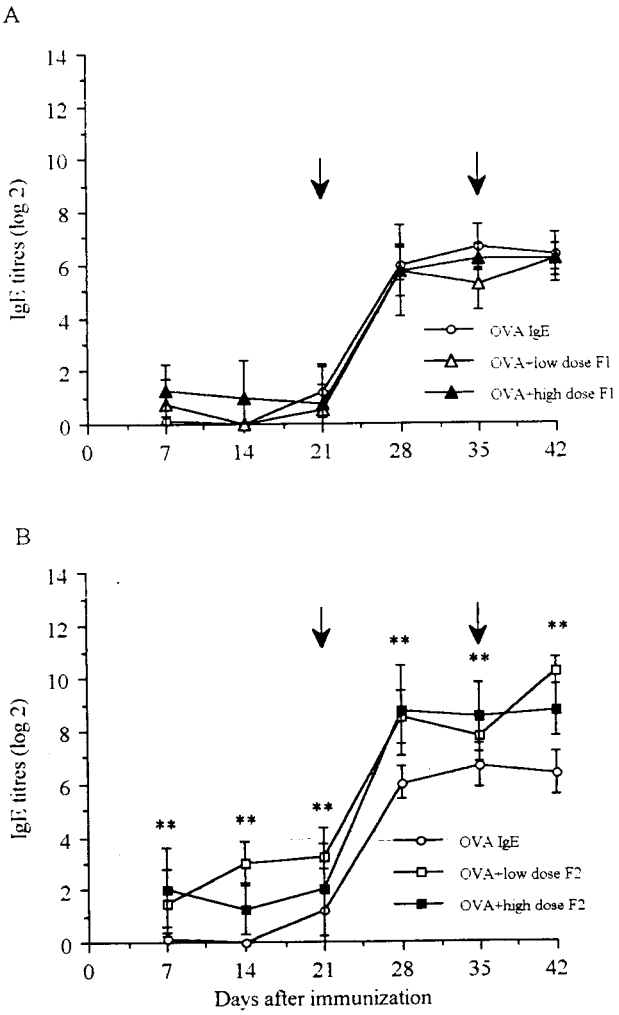

Figure 4. Kinetics of anti-OVA IgE antibody response modulated by artocarpin (F1) and jacalin (F2).
Swiss mice (10/group) were immunized with OVA in the absence or presence of 5 (low dose) and 50 µg (high dose) either F1 (A) or F2 (B) and on days 21 and 35 after primary immunization, booster injections were given in the same conditions (indicated by the arrows). The effects of 5 and 50 µg F2 (jacalin) was significant at all days with $P \leq 0.01$.

tory effect on the IgG1 response to OVA on the 35th and 42nd days. Artocarpin at a dose of 50 µg significantly stimulated anti-OVA IgG1 only on the 42nd day (Figure 3A). In addition, 5 µg jacalin up-regulated specific IgG1 synthesis at 28, 35 and 42 days. However, 50 µg of jacalin did not significantly stimulate IgG1 production at any time (Figure 3 B).

Concerning the effects of artocarpin (F1) and jacalin (F2) on the anti-OVA IgE response, artocarpin did not exert any effect on anti-OVA IgE synthesis (Figure 4A) whilst jacalin significantly enhanced primary and secondary responses, independently of the dose of this lectin (Figure 4B).The statistical analysis (ANOVA) did not reveal an interaction between the factor time and treatment. The difference between the means was evaluated by the Tuckey test with significance determined at the level of 99%.

DISCUSSION

Both lectins from *A. heterophyllus,* artocarpin and jacalin, have been used in immunological studies [1,2] although, somewhat conflicting interpretations have been reported concerning their mitogenicity. Indeed, this could be attributed to the diverse procedures used for isolating these lectins. Furthermore, jacalin has at least four different isoforms, which has been confirmed by the recent evidence of the existence of four cDNA clones encoding for this lectin [14]. Following the identification of a second D-mannose-specific lectin in the seeds of *A. heterophyllus,* artocarpin, the previously assigned role of jacalin as a T-cell mitogen was also found to be exerted by this lectin [15]. In fact, the number and content of lectins in jackfruit seeds is not completely known so far and a new lectin present in the globulin fraction has recently been described [8].

In the present study, in order to avoid ambiguities in their immunomodulatory effect we prepared artocarpin (F1) and jacalin (F2) by using the albumin fraction, purifying them on a guar gum column. Apart from the mitogenic properties that render the lectins versatile molecular probes useful as tools in immunological studies [16,17], other aspects such as a carrier capacity to induce antibody response against the TNP hapten attributed to jacalin [18] has been also considered to be of interest. The data in Figure 1 show that artocarpin (F1) and jacalin (F2) were able to induce specific antibody production on secondary immune response. These results show that these lectins are immunogenic by themselves. In addition, jacalin and artocarpin enhanced the antibody response to OVA, irrespective of their distinct carbohydrate specificity. Apparently, the stimulating effect of both lectins could not be attributed to the binding of the lectin to the antigen since jacalin which does not bind to the mannose residues of ovalbumin had the same modulatory effect as artocarpin that binds to this antigen (Figure 2). In spite of not being completely clarified, the modulatory effect of these lectins might be assigned to their capacity to induce cytokine synthesis since it has already been demonstrated for the secretion of IL-6 promoted by jacalin [6]. In reality, jacalin may exert its adjuvant effect on the *in*

vivo immunoglobulin synthesis by inducing macrophages to secrete interleukin-1 and interleukin-6 as it has already been described *in vitro* experiments. Furthermore, supernatant from jacalin-stimulated macrophages was shown to induce B cells to secrete IgM, IgG1, IgG2a, IgG2b, IgG3 and IgA [19].

In Figure 3 it can be seen that 5 µg of jacalin and artocarpin enhanced the OVA specific IgG1 response. On the other hand 50 µg of jacalin had no effect on this antibody synthesis. The mechanism whereby that low dose of jacalin and artocarpin stimulated IgG1 production was not studied. In a high dose of 50 µg these lectins seem to stimulate other Ig classes or IgG subclasses, since both lectins were able to enhance OVA specific Ig synthesis, as described above.

Our data showed that artocarpin had no effect on the anti-OVA IgE synthesis but that jacalin was able to enhance IgE synthesis (Figure 4). Such effect suggests that this lectin may, indirectly through IL-6, induce $CD4^+$ naive Th cells to differentiate into IL-4 Th2-producing ones that are known to regulate IgE production [20]. This hypothesis is supported by the fact that IL-6 directs the differentiation of IL-4-producing $CD4^+$ T cells *in vitro* [21]. Such a diverse mode of action of these two lectins confirms the versatility and usefulness of these lectins for immunological studies but more experiments are needed to clarify their mechanism of action.

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