Jacalin regulates IgA production by peripheral blood mononuclear cells

Aims: In IgA nephropathy, circulating immune complexes containing IgA1 are deposited on the glomerular mesangium, causing mesangial cell proliferation and acceleration of extracellular matrix production. The suppressive effect of jacalin, a galactose-binding lectin, on IgA production *in vitro* was determined. **Materials & methods:** Normal human peripheral blood mononuclear cells were stimulated with plate-bound anti-CD3 and Th2 stimulation, with or without jacalin. Regulatory and effector cell subsets were determined by flow cytometry, and immunoglobulin production by ELISA. **Results:** Jacalin increased the ratio of $CD4^+CD25^+CD152^+$ Tregs:effector T cells in peripheral blood mononuclear cell cultures 60-fold. This $CD4^+CD25^+CD152^+$ Treg increase may have inhibited Th2-stimulated IgA production by B cells. **Conclusion:** Immune tolerance induced by jacalin can suppress IgA production.

KEYWORDS: IgA nephropathy ■ jacalin ■ lectin ■ peripheral blood mononuclear cell ■ Treg

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IgA nephropathy (IgAN) is the most common form of glomerulonephritis and is characterized by elevated serum IgA. Deposition of circulating immune complexes (CICs) containing IgA on the glomerular mesangium causes the proliferation of mesangial cells and acceleration of extracellular matrix production in glomerulonephritis [1–5]. Although the etiology of IgAN is not fully understood, suggested causes are genetic defects resulting in aberrant lymphocyte class switching [6], food antigens [7] and influenza antigen-induced immune complexes [8]. Although IgAN patients have elevated circulating IgA, their IgG levels are normal, causing long-term imbalances in immunoglobulin (Ig) levels. As a result, IgA is bound by IgA-binding receptors on mesangial cells in the glomerulus, such as the transferrin receptor or IgA Fc receptor [3,9–11], which cause proliferation. In addition, IgA deposition on mesangial cells induces inflammation [10].

Of the two IgA subtypes, IgA1 is considered to make up the majority of IgA-containing CICs deposited on mesangial cells [4]. The IgA1 subtype contains sugars on the hinge region, which are typically absent in IgA2. The sugars are grouped into five types: ST and dST are normal sugar types, while T, Tn and STn are abnormal sugar types. Unlike in healthy individuals, circulating IgA1 in IgAN patients has a higher concentration of abnormal sugar types [12–16]. However, non-IgAN patients and healthy individuals also have IgA1 containing the abnormal sugar types [17]. In an earlier study [18], the present authors researched the sugar pattern on IgA in IgAN serum by using denatured jacalin; the ratio of abnormal sugars to normal sugars was 62.5:20.9% in IgAN serum and normal control serum, respectively.

Abnormal sugar types may relate to the pathological mechanism of IgAN; however, this is controversial. Furthermore, polymeric-IgA, which results from polymerization of IgA1 [19] and CIC containing IgA1 [3], has been investigated as an index of disease progression.

In addition to nephropathy treatment, it is necessary to treat elevated serum IgA1. Steroid pulse treatment [20] and tonsillectomy [16] are effective and reduce inflammation. However, long-term steroid treatment [21] and tonsillectomy are known to be immunosuppressive. Thus, there is need for a novel therapy without deleterious side effects.

The aim of this study was to identify a novel, safe, immunotherapeutic approach to regulate IgA production from B cells. Activated $CD4^+$ naive T cells (Th0) are differentiated into T-helper subsets (i.e., Th1, Th2 and Th17) or Tregs, depending on the local cytokine milieu [22]. Th1 and Th17 are effector cells involved in cytotoxicity, while Th2 effectors produce cytokines that stimulate Ig production from B cells. Tregs function to regulate the immune system [23–25] and can suppress the function of Th2 cells. Unlike cancer immunotherapies, which would require increasing effector T-cell function, autoimmune diseases such as IgAN can be treated by controlling effector cell function through Tregs.

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Jacalin, a lectin present in edible jackfruit seeds, binds to *O*-linked glycosides on IgA1. Jacalin has been found to have mitogen activity [26] and to act on B cells via IgA production directly [27]. However, these studies did not discuss its potential for IgAN treatment; moreover, the details of the immune mechanism are unknown for T cells, Tregs and B cells. Thus, on the basis of previous research [17,28,29], the present study clarifies the mechanism of jacalin-induced IgA production from B cells and aims to propose the application of jacalin in IgAN treatment.

Materials & methods

Purification of jacalin

Jacalin, a water-soluble protein, was extracted from the seeds of jackfruit as previously described [18]. Jackfruit seeds (10 kg) were crushed by a food processor and suspended in deionized water. The suspension was filtered through a membrane filter (pore size: 0.22 µm; Millipore, Billerica, MA, USA) for sterilization, and the filtrate was dialyzed against deionized water in a cellulose acetate tube (cut-off molecular weight: 10 kDa; Sanko Junyaku, Tokyo, Japan) at 4°C for 72 h. The dialyzed filtrate was freeze-dried to yield 24 g of crude powdered extract. The molecular weight of purified jacalin was measured using a high performance liquid chromatography system and a SW2000 column (Tosoh, Tokyo, Japan). The 15- and 60-kDa samples contained purified jacalin monosubunit (α-subunit) and tetrameric protein, respectively.

Samples & cell preparation

Blood and plasma samples were obtained from IgAN patients at Mie University Hospital (Japan), with their consent, and in agreement with the ethical guidelines of the medical department. A total of 30 plasma samples from IgAN patients and 22 from healthy volunteers were analyzed. Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood using the density-gradient solution Lymphoprep (Axis-Shield PLC, Scotland, UK) in a centrifugal separator (Hitachi, Tokyo, Japan) [30]. Heparinized blood (15 ml) diluted in an equal volume of saline solution was added to the Lymphoprep tube and spun at 800 × g for 10 min to separate the PBMC fraction. $CD4^+$ T-helper cells and $CD19^+$ B cells were isolated from PBMCs using antibody-coated magnetic beads (Miltenyi Biotech, Bergish, Germany) [31]. PBMCs (10^7 cells/80 µl) suspended in phosphate-buffered saline (PBS) containing 1 mM ethylenediaminetetraacetic acid and 0.5% bovine serum albumin (BSA; Sigma-Aldrich, MO, USA) were incubated with CD4 or CD19 microbeads (Miltenyi Biotech) at 4°C for 15 min. After incubation, the cell suspension was centrifuged at 600 × g for 10 min; the cell pellet was resuspended with PBS containing 1 mM ethylenediaminetetraacetic acid and 0.5% BSA. $CD4^+$ cells and $CD19^+$ cells were collected through the LS-column of a magnetic activated cell sorting separator (Miltenyi Biotech).

Cell culture

Isolated $CD4^+$ T cells were suspended at a concentration of 10^6 cells/ml in RPMI 1640 medium (Sigma-Aldrich; supplemented with L-glutamine, 1% fetal bovine serum (FBS), penicillin and streptomycin). For Th2 differentiation studies, cells were incubated in 24-well plates coated with anti-CD3 antibodies (1 µg/ml in lieu of APCs) at 37°C for 2 h; IL-2 (20 ng/ml) and IL-4 (20 nM/ml) were then added, and the plate was incubated at 37°C (5% CO_2) for 5 days. For Ig production, PBMCs or $CD19^+$ cells were suspended in RPMI 1640 (2×10^5 cells/ml) in 24-well plates under two stimulation conditions: anti-CD40 (0.5 µg/ml) and IL-4 (5 µg/ml) at 37°C for 7 days (i.e., T-cell dependent Ig production) and IL-4 (5 µg/ml) alone at 37°C for 7 days (i.e., T-cell independent Ig production). Where required, jacalin was added to a final concentration of 10 µg/ml in each well.

Flow cytometry

The differentiation of PBMCs, $CD4^+$ T cells and $CD19^+$ B cells by jacalin was evaluated by flow cytometry using FACSCalibur (Becton Dickinson, NJ, USA). PBMCs or T cells were suspended in 1 ml of PBS containing 2% FBS and stained with 20 µg/ml of anti-CD25-fluorescein isothiocyanate antibody (Ab; clone B1.49.9; Beckman Coulter, CA, USA) and anti-CD152-PE Ab (clone L3D10, Beckman Coulter, CA, USA; 20 µg/ml; anti-CTLA-4 Ab) to measure Tregs. To quantify the B-cell subclasses, B1 and B2, PBMCs or B cells were suspended in 1 ml of PBS containing 2% FBS and stained with 20 µg/ml of anti-CD19-APC Ab (clone LT19, AbD Serotec, Oxford, UK), 20 µg/ml of anti-CD5-PECy7 Ab (clone 53–7.3; Santane Cruz Biotechnology, CA, USA) or 20 µg/ml of polyclonal anti-IgA-RPE Ab (AbD Serotec).

ELISA

The concentration of IgA, IgG, TGF-β and CICs in serum samples and cell culture supernatants were measured by ELISA. For TGF-β measurement, samples were activated with 0.2N HCl for 10 min and measured by the human TGF-β DuoSet (R&D Systems, MN, USA), with unknown sample concentrations extrapolated from the standard curve. IgA and IgG were measured with human IgA or IgG ELISA Quantitation kits (10,000-fold dilution; Bethyl, TX, USA) and concentration was determined from the standard curve with the international standard plasma (IRMM ERM-DA470; ReCCS, Tokyo, Japan). Antihuman IgA or IgG were added to a 96-well plate (100 µl/well) and incubated for 1 h to coat the plate. Plates were washed with PBS containing 0.05% Tween 20 and incubated for 30 min with a 0.5% BSA solution for blocking. Samples were added to the wells (100 µl/well) and incubated for 1 h. After washing with PBS containing 0.05% Tween 20, horseradish peroxidase (HRP)-labeled anti-IgA/IgG antibody was added and incubated for 1 h. Plates were washed once more, and a color reagent, *O*-phenylenediamine, was added and incubated for 10 min. Next, 2N sulfuric acid was added and the absorbance was measured at 490 nm using a microplate reader (Bio-Rad Laboratories, CA, USA). Concentrations of the proteins were calculated from the curves prepared with reference to the standard molecules. In case of CICs–ELISA, the base of the IgA ELISA was used as described above, except for the use of HRP-labeled anti-IgG to detect CICs.

Statistical analysis

All data are expressed as the mean ± standard deviation (SD). Statistical significance was determined at a p-value of <0.05. Calculations were performed using the statistical package StatView 5.0 (SAS Institute, NC, USA).

Results

Serum IgA & IgA$^+$ B cells

Total serum IgA concentration was measured in healthy control individuals (n = 22) and IgAN patients (n = 30; FIGURE 1A) by ELISA. Average IgA concentrations were significantly higher in IgAN patients than control individuals (349 ± 116 and 248 ± 42 mg/dl, respectively; mean ± SD; $p < 0.01$). CIC concentration was significantly higher in IgAN patients than control individuals (107 ± 33 and 61 ± 21 mg/dl, respectively; mean ± SD; $p < 0.01$). In the serum of IgAN patients, the concentration of IgA, which exists as an immune complex, was also higher than that in the serum of healthy persons (FIGURE 1B).

We next stained freshly isolated PBMCs with anti-CD5 and -CD19 to analyze B-cell populations by flow cytometry. We described that CD5$^+$CD19$^-$ populations were classified as T cells (activated or undifferentiated). B1 cells are very important lymphocytes involved in autoimmunity. Recently, until its marker was clearly established, CD5 was used as the B1 cell marker. In an earlier report [32], CD5$^+$ cells with the marker profile CD20$^+$CD27$^+$CD43$^+$ were found to account for 75% of human B1 cells.

Then, in this study, we changed to CD5$^+$cells from expression of B1 and B2 cells. The ratio of total B cells (B1 and B2 cells) in lymphocytes was similar in control individuals and IgAN patients (~20%; data not shown). However, the percentage of IgA$^+$ CD5$^+$CD19$^+$ and CD5$^-$CD19$^+$ cells was higher in IgAN patients (FIGURE 1C & D). IgAN patients had 61 ± 14% IgA$^+$ CD5$^+$CD19$^+$ cells, while control individuals had 34 ± 7% IgA$^+$ CD5$^+$CD19$^+$ cells (n = 3 per group; $p < 0.01$). Similarly, IgAN patients had elevated IgA$^+$ CD5$^-$CD19$^+$ cells compared with control individuals, with 40 ± 5.5% and 20 ± 5.5%, respectively (n = 3 per group; $p < 0.01$).

Effect of jacalin on PBMC TGF-β production

We determined that jacalin binds to nearly 100% of purified CD4$^+$ T cells (FIGURE 2). Next, we investigated the effect of jacalin on *in vitro* cell culture. Total PBMCs were cultured *in vitro* under Th2 polarization conditions and TGF-β production was measured after 5 days (FIGURE 3). Since one of the main cytokines produced by Treg is TGF-β, this cytokine was used as a marker of Treg differentiation [33]. TGF-β production was significantly increased under Th2 polarization conditions (mean: 1950 pg/ml) compared with media alone. The addition of jacalin to cell culture further increased TGF-β production (2500 pg/ml). This is indirect evidence that the percentage of Treg was increased by jacalin.

Effect of jacalin on Th2 differentiation in purified CD4$^+$ T cells

Purified CD4$^+$ cells were cultured under Th2 stimulation conditions with or without jacalin, as described above. The expression of CD25 and CD152 was measured every day for 5 days and the change in CD4$^+$CD25$^+$CD152$^-$,

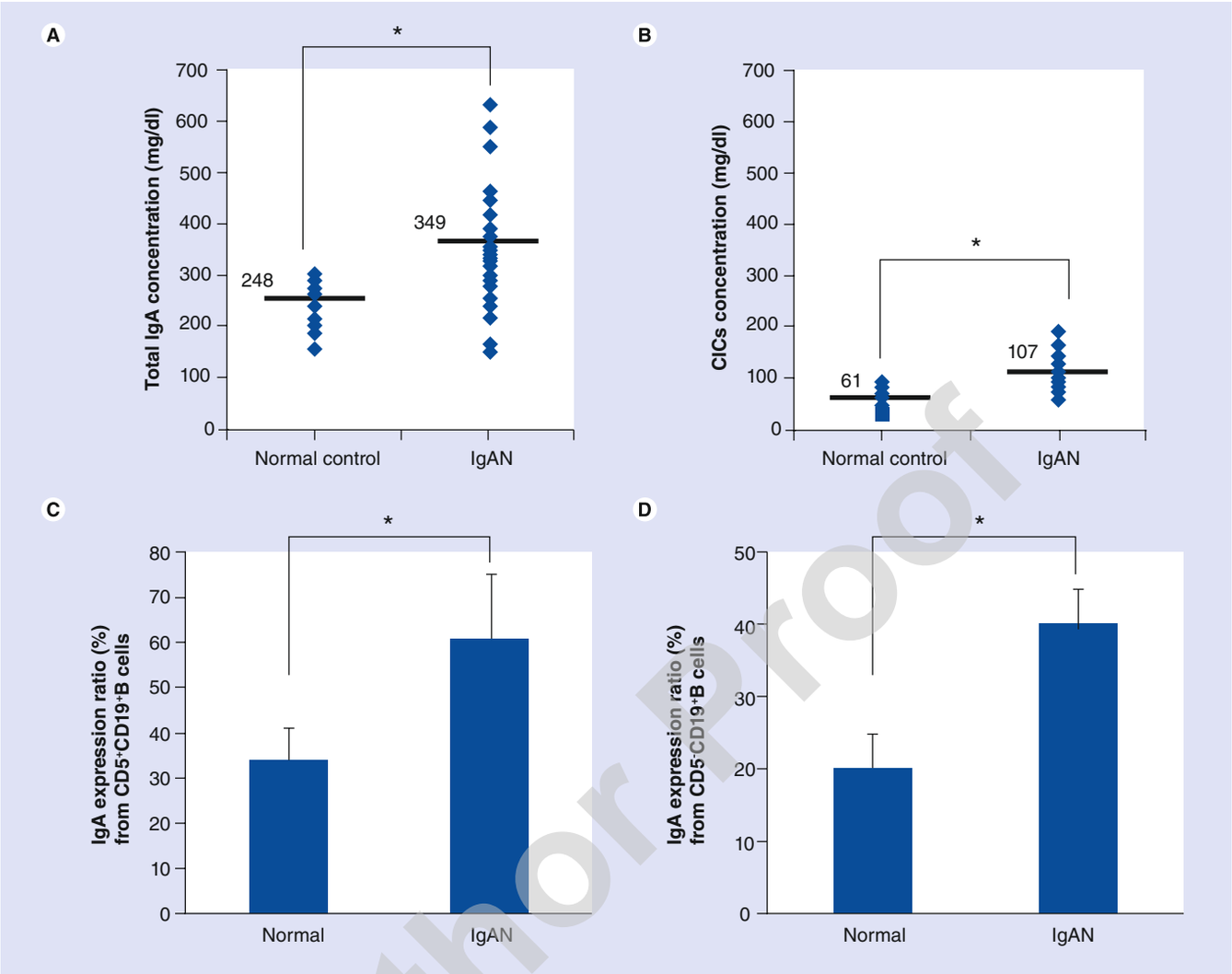

Figure 1. Circulating IgA and IgA$^+$ B cells are elevated in IgA nephropathy patients. (A) Total IgA concentration in control (248 ± 42 mg/dl; n = 22) and IgAN (349 ± 116 mg/dl; n = 30) serum. **(B)** Circulating immune complex concentration in control (61 ± 21 mg/dl; n = 22) and IgAN (107 ± 33 mg/dl; n = 30) serum. **(C)** IgA expression on the surface of CD5$^+$CD19$^+$ B cells **(D)** and CD5$^-$CD19$^+$ B cells. Data presented as mean ± SD.
*p < 0.01.
CIC: Circulating immune complex; IgAN: IgA nephropathy; SD: Standard deviation.

CD4$^+$CD25$^-$CD152$^+$ and CD4$^+$CD25$^+$CD152$^+$ T cells was plotted longitudinally (Figure 4). CD152, also referred to as CTLA-4, is known to be expressed on FOXP3$^+$ cells, a lineage marker of Tregs [33]. Jacalin addition resulted in higher levels of CD4$^+$CD25$^+$CD152$^+$ cells over all 5 days. CD4$^+$CD25$^+$ cells, which may be Tregs or activated T cells, and CD4$^+$CD152$^+$ cells were both increased. CD4$^+$CD25$^-$CD152$^-$ cells represent effector cells (Th1, Th2 and Th17). In Figure 5, the ratio of regulatory cells (i.e., CD4$^+$CD25$^+$, CD4$^+$CD152$^+$ and CD25$^+$CD152$^+$) to effector cells was plotted longitudinally over the 5-day culture. Values were normalized to the control media-alone condition to equalize cell numbers. With the addition of jacalin, there was a higher ratio of Tregs:effectors, peaking at 0.6 at day 3. By contrast, Th2 conditions alone did not alter the regulatory:effector T-cell ratio.

Effect of jacalin on PBMC production of IgA

We next determined the effect of jacalin on IgA and IgG production. PBMCs were cultured *in vitro* under Th2-stimulating conditions and IgA production was measured by ELISA (Figure 6A). Although IgA production increased under Th2 stimulation compared with media alone (1.1-fold higher; p < 0.01), Th2 stimulation plus jacalin resulted in IgA production similar to the control condition. There was no change

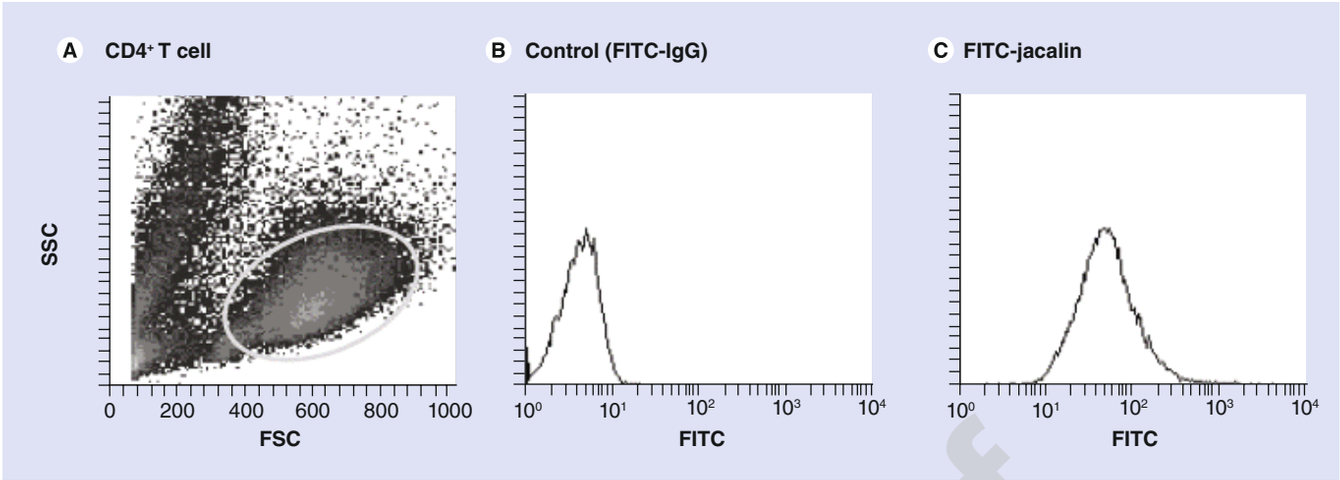

Figure 2. Jacalin-binding capacity of CD4⁺ helper T cells. (A) Representative flow cytometry plot of the lymphocyte gate on purified CD4⁺ T cells. **(B)** Fluorescence intensity of FITC-IgG isotype (control) on gated CD4⁺ T cells. **(C)** Fluorescence intensity of FITC-jacalin on gated CD4⁺ T cells.
FITC: Fluorescein isothiocyanate; FSC: Forward scatter; SSC: Side scatter.

in IgG production under any of the conditions (data not shown). Therefore, the ratio of IgA:IgG produced is reflective of the change in IgA production (Figure 6B; normalized to control).

Ig production by stimulated CD5⁺ CD19⁺ & CD5⁻CD19⁺ B cells

It is known that IgA and IgG production can occur in both B2 cells with helper T-cell activation and in B1 cells in a helper T-cell-independent manner [34]. We next determined the ability of purified CD19⁺ B cells to produce Ig in a T-cell independent capacity (i.e., IL-4 stimulation alone through B1 cells), as well as in a T-cell-dependent capacity (i.e., IL-4 and anti-CD40 through B2 cells). B cells were cultured for 5 days under these conditions and Ig concentrations were measured in the supernatants. While IL-4 with anti-CD40 stimulation increased IgA concentration over media alone, the addition of jacalin seemed to abrogate this effect slightly (Figure 7A). The ratio of IgA:IgG reflected the changes in IgA production (Figure 7B). A similar trend was observed with IL-4 stimulation alone; however, it was not significant (Figure 7C & D).

Discussion

Mechanism of IgA production

Serum IgA concentrations are elevated in IgAN patients, with over 50% of patients having levels above the control range. Excess IgA is deposited in the glomerulus, exacerbating the disease. Therefore, a treatment to reduce IgA levels in such patients would be highly beneficial. In this study, it was found that the ratio of lymphocytes and B cells was similar in IgAN patients and healthy individuals; however, there were greater numbers of IgA⁺ CD5⁺CD19⁺ B and CD5⁻CD19⁺ cells in IgAN patients. This observation is a likely factor behind the elevated IgA serum

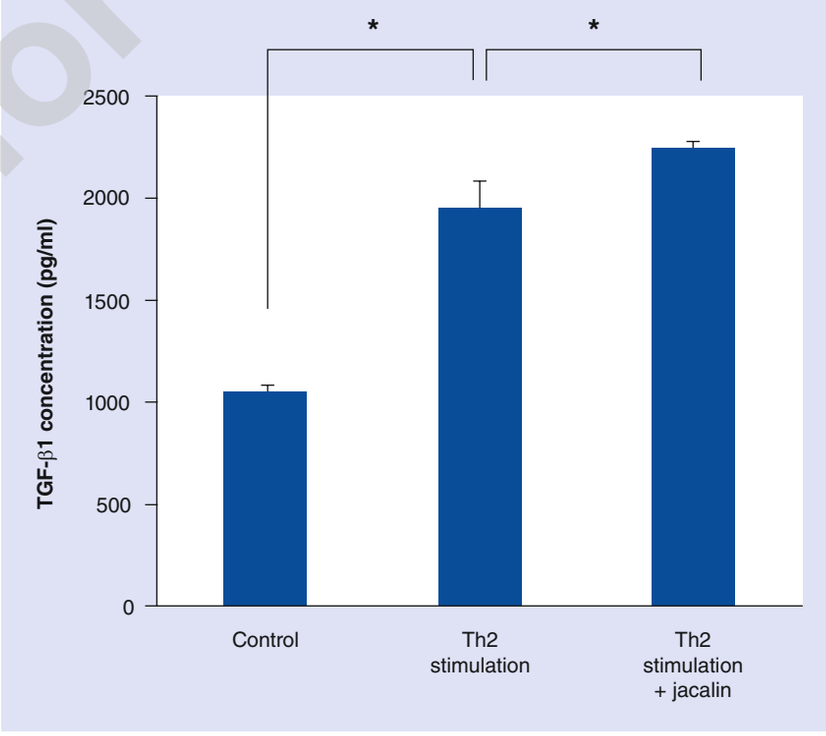

Figure 3. Jacalin increases TGF-β production *in vitro*. Peripheral blood mononuclear cells were cultured for 5 days with plate-bound anti-CD3, with media alone or under Th2 stimulation (exogenous IL-2 and IL-4). The effect of jacalin addition was determined by measuring TGF-β production by ELISA; n = 3. *p < 0.01.

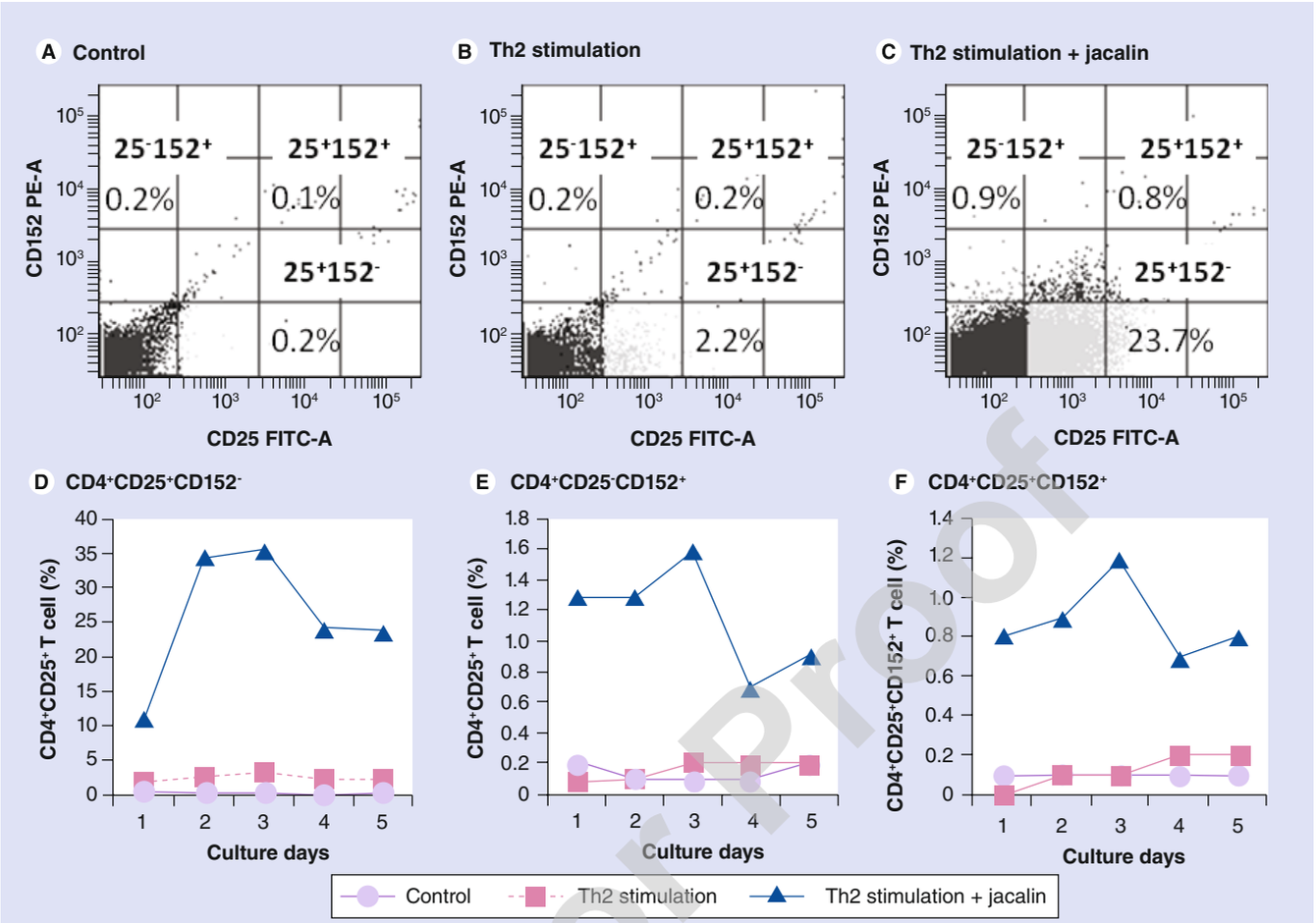

Figure 4. Jacalin induces Tregs under Th2 stimulation conditions. (A–C) Representative CD25 and CD152 staining of 5 × 10^5 purified $CD4^+$ cells at day 5 under control conditions (media alone), Th2 stimulation and Th2 stimulation with jacalin. **(D–F)** Change over time in percentage of T-cell subsets under control, Th2 stimulation and Th2 stimulation plus jacalin conditions.

concentrations in patients. The imbalance in B-cell classes may be due to abnormal helper T-cell induction of class switching in B cells. Although IgA^+ cells do not always produce antibodies, B1 cells, which are known to produce antibodies in mucosal immunity in a T-cell-independent manner, mainly produce IgA [35]. We found that IgA production from B cells in IgAN patients increased 1.5 times compared with that in healthy individuals. Considering that Th2 cells produce cytokines that stimulate antibody production, we have proposed a model in order to explain IgA production in IgAN patients (Figure 8). To date, the antigens responsible for the pathogenesis of IgAN have not been determined. However, it can be assumed that there is a defect in Th2 stimulation of antibody production. Upon antigen stimulation, undifferentiated helper T cells mature into Th2 cells, which produce IL-4 and IL-2. These cytokines promote B2 differentiation, which secrete additional cytokines and mature into antibody-producing plasma cells. Concurrently, in the presence of IL-4, B1 cells transform into plasma cells and produce IgA. Some of the IgA produced comprise IgA1 with abnormal sugar chains [17], which are deposited on the glomerulus and exacerbate disease. Treg types, such as Th3 and Tr1 [36], vary in the type of cytokines they produce, with many of their functions still unclear. However, Tregs are known to suppress Th2 cells and autoimmune responses. Rather than removing the allergy antigen, inhibition of Th2-induced antibody production may be achieved by preventing the differentiation of Th0 cells into Th2 cells.

Suppression of IgA production by jacalin

The aim of this study was to determine the suppressive effect of jacalin on B-cell IgA production. We found that jacalin decreases Th2-induced IgA production *in vitro*. Since

IgG production remained unchanged, the IgA:IgG ratio was reflective of the change in IgA production. There are several possible mechanisms to explain the effect of jacalin. Jacalin has a high affinity for $CD4^+$ T cell, and although it is believed that jacalin binds the sugar chains on the cell surface, the absolute target of jacalin is unclear. Jacalin is considered to recognize sugar types such as T (CD176: Gal β1-3GalNAc α1-O-R), Tn (CD175: GalNAc α1) and STn (CD175s: NeuAc α2-6GalNAcα1-O-R). Although these sugar antigens exist on the surface of lymphocytes [37,38], their functions are unknown. Therefore, the specific effect of jacalin due to binding these sugars remains unclear.

The amount of TGF-β produced by Tregs was increased by the addition of jacalin, implying that Th2 differentiation was decreased and Treg suppression was increased. Under these conditions, there would be a decrease in B2 differentiation. In support of this, IgA production by B cells was suppressed with the addition of jacalin, implying that jacalin suppressed the differentiation of B cells to plasma cells. It is known that IgAN patients have elevated levels of B1 cells compared with healthy control individuals [35]. B1 cells of the mucosal immune system are considered to function in a T-cell-independent manner. The effect of jacalin on B1 cells is therefore of great interest; however, we did not find that jacalin significantly suppressed IgA production of purified B cells. The effect of jacalin on IgA production in PBMC cultures is therefore likely due to increased TGF-β production from Tregs.

Accordingly, a second possible mechanism for jacalin is through Treg induction. FOXP3 is a lineage marker of Tregs, which also express $CD25^+$. CD152, also known as CTLA-4, is present on Tregs and can induce Th0 cells to

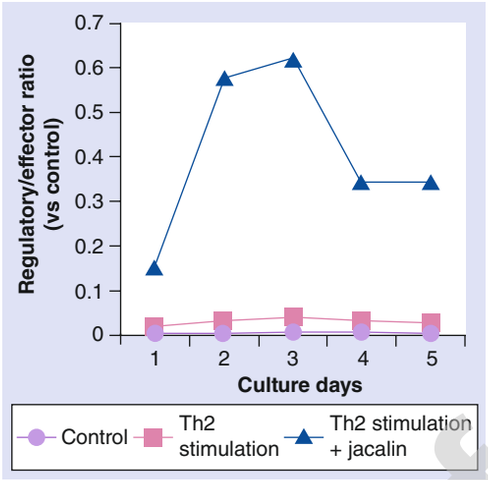

Figure 5. Longitudinal analysis of the regulatory:effector T-cell ratio under Th2 stimulation with the addition of jacalin. Regulatory T-cell percentages ($CD4^+25^+$, $CD4^+CD152^+$ and $CD25^+CD152^+$) were compared with effector ($CD4^+CD25^-CD125^-$) cells over the 5-day stimulation and cell number was normalized to the control.

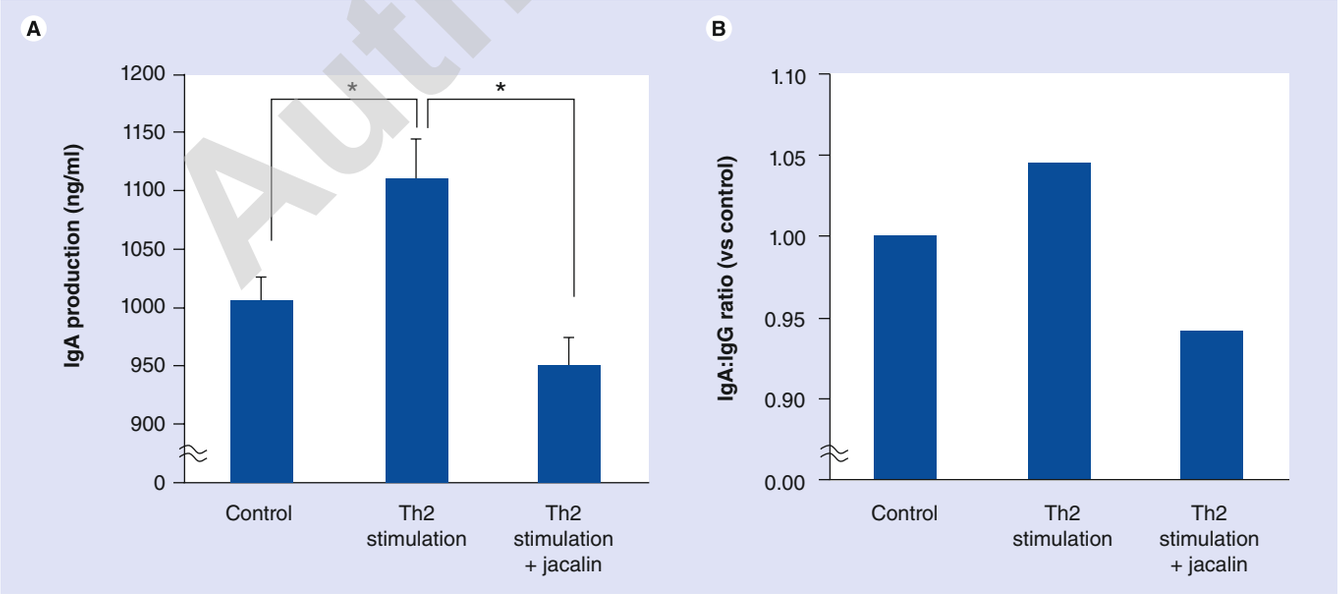

Figure 6. The effect of jacalin on peripheral blood mononuclear cells' production of IgA. (A) IgA production at day 5 from peripheral blood mononuclear cells in medium alone, Th2 stimulation conditions or Th2 conditions plus jacalin. **(B)** The IgA:IgG ratio produced from cultured peripheral blood mononuclear cells, normalized to control. Data presented as mean ± standard deviation; n = 3. *$p < 0.01$.

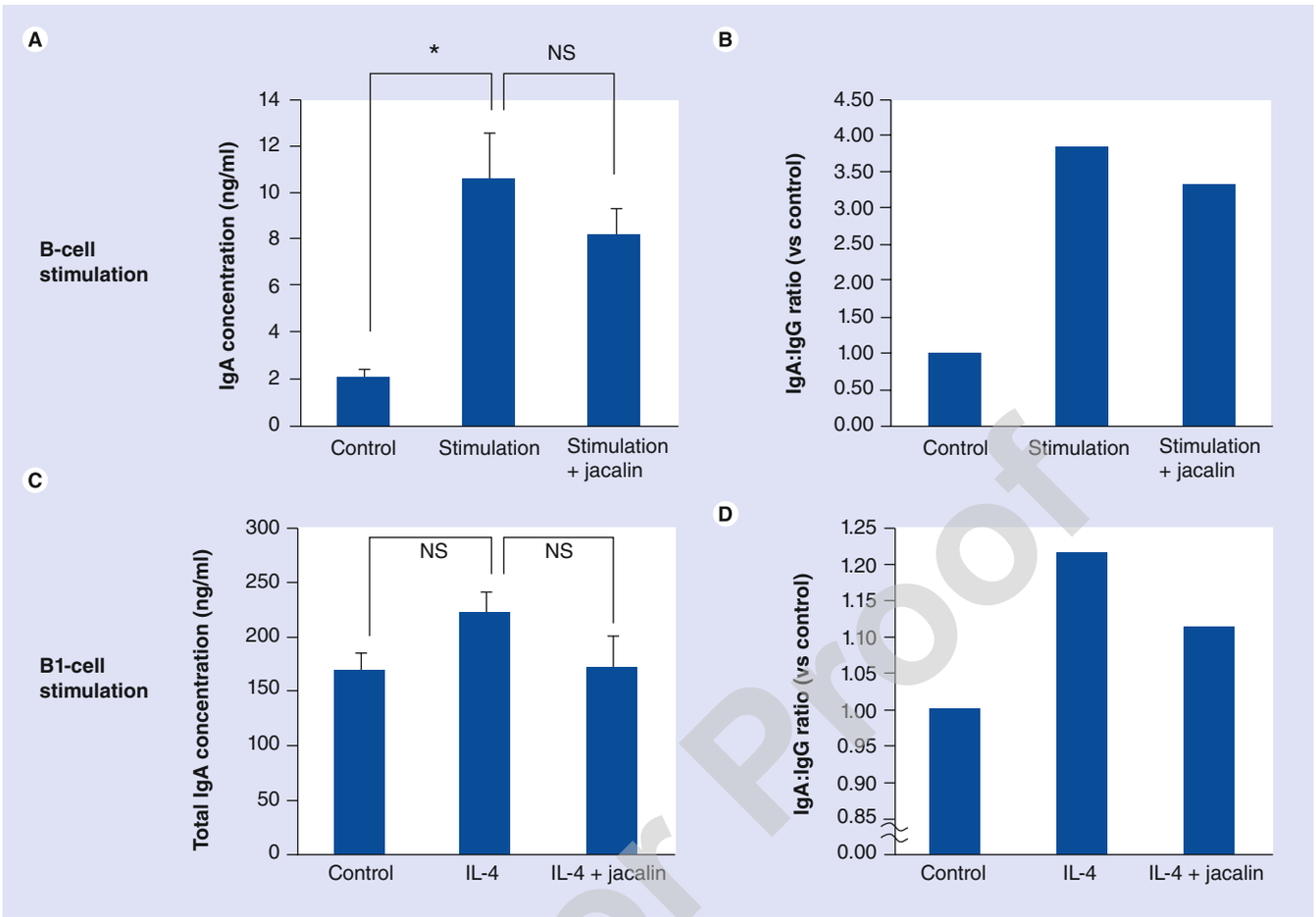

Figure 7. The effect of jacalin on IgA production by B1 and B2 cells. (A) IgA production at day 5 from B cells stimulated with IL-4 and anti-CD40, with or without jacalin and **(B)** the IgA:IgG ratio. **(C)** IgA production from B cells stimulated with IL-4 alone, with or without jacalin, and **(D)** the IgA:IgG ratio. IgA:IgG ratios are normalized to control. Data presented as mean ± standard deviation; n = 3.
*$p < 0.01$.
NS: Not significant.

differentiate into Tregs by signaling through B7 [39,40]. Accordingly, CD25 and CD152 were used as markers for Tregs in this study.

$CD25^+CD4^+$ Tregs are cell groups that control superfluous immune responses such as in an autoimmune disease and allergy. Tregs are highly expressed on CD152 (CTLA-4) and FoxP3 is known to control this expression [41]. Tregs control the immune response by lowering the activation ability of other T cells by APCs, for which CTLA-4 is indispensable. Thus, owing to its inhibitory effect, we chose Tregs as a marker system.

However, the general marker of Tregs is known to be FoxP3. Tregs have a constant, high expression of CD152 (CTLA-4) and it is known that Foxp3 controls the expression of CTLA4. We distinguished the regulatory cell in this study from the Tregs that expressed FoxP3. $CD4^+CD25^+CD152^+$ Tregs were expressed in this study.

Treg activation is known to require CTLA-4 and CD28 signaling [42]. However, CD28 was not added to the *in vitro* cell culture experiments. It is possible that jacalin induces an effect similar to that of CD28.

To quantify the tolerogenic effect of jacalin, we determined the ratio of regulator to effector T cells. Jacalin has been considered an adjuvant, which implies that it induces the activation of the immune system, particularly the activation of T cells. However, at the same time, jacalin induces the upregulation of $CD4^+CD25^+CD152^+$ Tregs and exerts an inhibitory effect on IgA synthesis.

It found that jacalin increased $CD4^+CD25^+CD152^+$ Treg numbers without increasing effector T-cell numbers, leading to an elevated regulatory:effector ratio. Therefore, jacalin might function by two different mechanisms: the induction of $CD4^+CD25^+CD152^+$ Tregs from Th0 cells and the inhibition of plasma cell

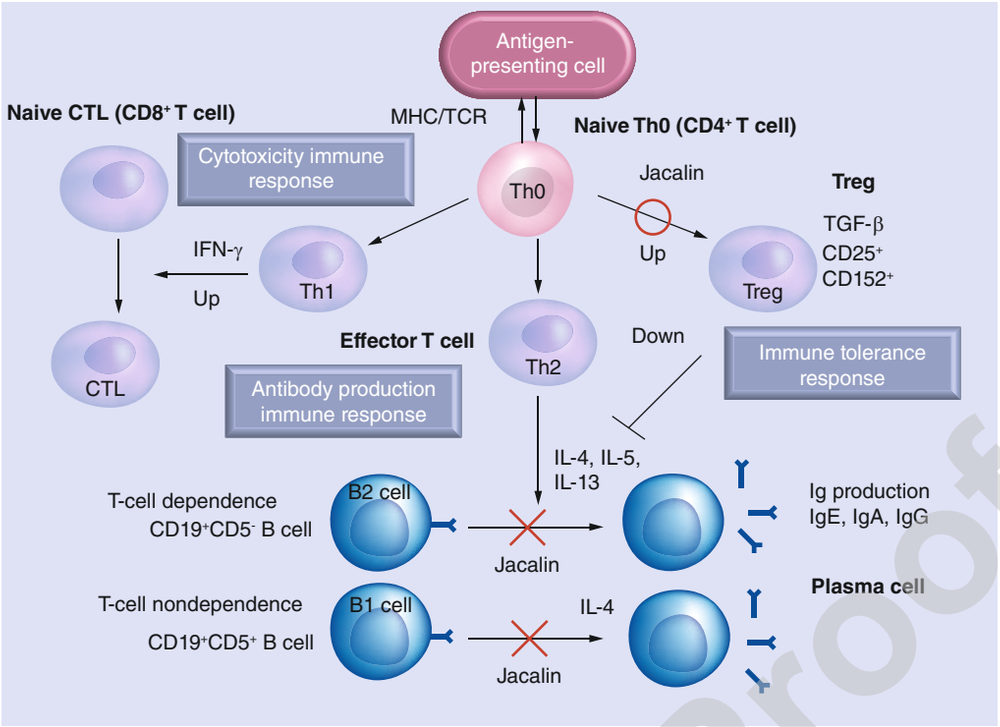

Figure 8. Jacalin creates a tolerogenic state, which regulates IgA production. In this model, it is hypothesized that jacalin induces naive T cells to differentiate into Tregs, which inhibit the Th2-induced humoral immune response. This leads to a decrease in plasma cell differentiation from B cells.
CTL: Cytotoxic T lymphocyte; TCR: T-cell receptor; Th0: Naive T cell.

differentiation. The details of these mechanisms require further investigation.

Therapeutic potential of jacalin

Reducing circulating IgA levels in IgAN patients is necessary to decrease IgA deposits in the glomerulus. Current treatments utilizing steroids are suboptimal due to their potential side effects. However, autoimmune diseases may be treated by immunotherapies, which correct defects in the immune system. In the case of IgAN, promoting immune tolerance to reduce IgA production would be an ideal solution. The goal of this study was to develop a novel treatment that showed efficacy *in vitro*. We suggest apheresis treatment with jacalin as an immunostimulant as a possible medical treatment for direct stimulation of immune cells in the blood.

Other diseases such as alcoholic cirrhosis [43], connective tissue disease [44], lymphoproliferative disease [45] and chronic hepatitis [46] also have elevated circulating IgA. IgA can also be deposited in the glomerulus of IgAN patients with normal concentrations of circulating IgA. The relationship between IgA receptors and IgA sugar types have been reported as important factors in disease pathogenesis. IgA with abnormal sugar types will aggregate, causing the formation of CICs containing IgA [47]. These abnormal sugar chains are considered a defect in the glycosylation system [48]. Deposition of CICs on mesangial cells promotes proliferation and the production of inflammatory cytokines, which attract immune cells, eventually leading to collagen production and nephropathy [49]. It was hypothesized that jacalin inhibits CIC formation by binding to abnormal sugar chains in IgA. Herein, it was demonstrated that jacalin increased $CD4^+CD25^+CD152^+$ Tregs and reduced IgA production under Th2 stimulation conditions. These findings highlight the potential use of jacalin as a treatment for IgAN and other diseases with elevated production of IgA.

Conclusion

In this study, we show that jacalin can suppress IgA production *in vitro*. Jacalin promoted differentiation of Tregs and suppressed differentiation of plasma cells from B cells. Taken together, our findings indicate that jacalin has the potential to be a safe immunotherapeutic approach for the treatment of IgAN.

Future perspective

Modern advances allow measurement of the concentration and sugar-chain make-up of IgA. Moreover, research on an early detection method for IgAN is progressing. Due to these advances, verification of the immunotherapeutic benefits of jacalin and determination of its mechanism of action are feasible. The development of an effective therapy for IgAN will positively impact the number of patients with renal failure.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

IgA is a marker of disease

- Elevated serum IgA levels are common in IgA nephropathy and can exacerbate disease.
- IgA nephropathy patients have elevated numbers of circulating IgA$^+$ B1 and B2 cells.

Jacalin is a novel immunoregulatory agent

- Jacalin is capable of binding to CD4$^+$ T cells and promotes TGF-β production in *in vitro* peripheral blood mononuclear cells (PBMCs) stimulated with IL-2 and IL-4 (Th2 stimulation conditions).
- In purified CD4$^+$ cells, the addition of jacalin to Th2 stimuli causes an increase in CD4$^+$CD25$^+$CD152$^+$ Tregs compared with Th2 stimuli or media alone.
- In purified CD4$^+$ cells, 5-day cell culture with jacalin and Th2 stimuli results in a higher Treg:effector T-cell ratio than Th2 stimuli or media alone.
- In PBMCs, jacalin reduces Th2-stimulated IgA production to nonstimulated levels, but has minimal effect on purified B cells cultured under IgA-inducing conditions.

The potential for a safe immunotherapy

- Jacalin induced Tregs *in vitro*, which inhibited Th2-mediated B-cell differentiation and antibody production.
- Jacalin, a naturally occurring lectin, is capable of reducing IgA production from human PBMCs *in vitro* and has the potential to be a safe immunotherapy.

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