Journal of Clinical Laboratory Analysis 3:244–251 (1989)

Jacalin: A New Laboratory Tool in Immunochemistry and Cellular Immunology

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In recent years, a new lectin—jacalin—has raised the interest of immunologists because of its original properties with respect to human immunoglobulins and lymphocytes. Its structure and carbohydrate binding specifity are now well documented, and it can be purified easily from jackfruit seeds by ion exchange or affinity chromatography. The binding and precipitating specificities of jacalin with heavy chains of human immunoglobulins allow its use as a diagnostic (IgA subclass typing) and preparative tool (purification of IgA and IgD, removal of IgA from biologic samples and preparations). Other possible applications of jacalin's binding properties also can be envisaged. In addition, the lectin displays a mitogenic activity specific for human CD4 T-lymphocytes; consequently, the proliferative response induced by jacalin appears to represent a new and interesting assay for a functional study of CD4 cells, with obvious applications in primary and acquired, especially AIDS, immune deficiency states.

Key words: lectins, human immunoglobulins, human lymphocytes, polyclonal activation

INTRODUCTION

Lectins are proteins of nonimmune origin and are widely present in vegetables and animals. They were first described as able to precipitate certain serum components (1) and then as agglutinating various cell types, including erythrocytes, leucocytes, tumor cells, bacteriae, and viruses. These various agglutinin activities share the general property of being inhibited by specific monosaccharides or oligosaccharides, which suggests that they are mediated by membrane glycoproteins or glycolipids. After Nowell demonstrated that phytohemagglutinin (PHA) from *Phaseolus vulgaris* induces blastic transformation of normal lymphocytes (2), the mitogenic property of a variety of lectins was demonstrated; several of these lectins are commonly used today because of their activity on lymphocytes, including PHA, concanavalin A from *Canavalia ensiformis*, pokeweed mitogen from *Phytolacca americana*, and many others.

The first demonstration of a potential interest of agglutinin(s) extracted from jackfruit (*Artocarpus heterophyllus*) seeds in immunology was provided by Bunn-Moreno and Campos-Neto (3). Later, immunologists preferred the term *jacalin* to *jackfruit seed agglutinin*, which was used by others. Jacalin first received attention because of its mitogenic activity. The first report concluded that crude extract from jackfruit seeds (JCE) can stimulate in vitro human lymphocyte (mostly T cell) proliferation and immunoglobulin (Ig) production by human B cells (3). Somewhat contradictory results concerning the proliferative response of human lymphocytes to JCE have been published (4,5), but the main interest of jacalin began with demonstration of its reactivity with human IgA by Roque-Barreira and Campos-Neto (6), whose interest was stimulated by the finding of important precipitates in culture media containing jacalin and calf serum. Gel double-diffusion studies showed that, among human serum proteins, only IgA and a few α 2-globulins were precipitated by JCE. The well-known difficulties of purifying serum IgA or eliminating IgA from certain preparations (in particular, IgG preparations for therapeutic use) led us to study the properties of jacalin and their possible immunological applications. The present report reviews current knowledge on the chemical nature and binding properties of jacalin and examines its mitogenic activity which, although much less studied, should raise a new interest.

Received December 29, 1988; accepted January 4, 1989.

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Abbreviations used: PHA, phytohemagglutinin; JCE, jackfruit seed crude extract; Ig, immunoglobulin; DEAE, diethylaminoethyl; Mr, molecular weight; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PBMC, peripheral blood mononuclear cells.

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CHEMICAL CHARACTERIZATION

The first immunological studies performed with jacalin were in fact done with JCE obtained by steeping grounded seeds in phosphate-buffered saline for 24 hours (3,4) or with a partially purified preparation (7,8). Discrepant results of certain studies of reactivity with Ig (9) and effects on lymphocytes (see below) of such jacalin preparations pointed out the need for a strict characterization of this lectin. In fact, the first biochemical study of jacalin, performed by Moreira and Ainouz (10), was practically ignored by immunologists. Using jackfruit seeds collected in Brazil, these authors showed the heterogeneity of jacalin's electric charge, which the present authors confirmed using seeds from the Indian Ocean island La Réunion and also from Brazil (9,11). Diethylaminoethyl (DEAE) chromatography of JCE first yields two major fractions (F1 and F2) with comparable capacities of precipitating human IgA; both contain jacalin (defined as IgA-binding molecules made up of two subunits; see below) only. The following fraction (F3) is composed of several proteins migrating in the α 2-globulins by standard zone electrophoresis; the last fraction to be eluted (F4) has a yellow-brown color and migrates like serum albumin.

Jacalin, purified by ion exchange or IgA-affinity chromatography, is formed of two types of noncovalently linked glycosylated and unglycosylated subunits with apparent molecular weights (Mr) of 11.25 to 12 kD and 14.7 to 15 kD (10–12), respectively. The amino acid composition of these two subunits is very similar (Table 1). The different native forms of this lectin in fractions F1 and F2, termed *isolectins* by Moreira and Ainouz (10), have the same size as shown by gel filtration under nondissociating conditions (Mr about 50 kD), but they differ in the proportion of both types of subunits: the unglycosylated/glycosylated subunit ratio is 2.2 to 2.9 in F1 (which contains the bulk of jacalin) and 4.7 to 6.8 in F2 (Fig. 1). The fraction F3 contains a notable proportion of the lectin (as well as F4 to a lower degree) that is easily evidenced by Western blotting using rabbit antijacalin antibodies (Fig. 2). Analysis of jacalin purified by affinity chromatography from F3 showed that the subunit ratio in this fraction is sim-

TABLE 1. Amino Acid Composition of Subunits of Jacalin

	Amount from analysis rounded to the nearest integer	
Amino acids	Nonglycosylated subunit (mole/mole subunit)	Glycosylated subunit (mole/mole subunit)
Lys	7	7
His	1	1
Arg	2	2
1/2 Cys	0	0
Asp	10	11
Thr	8	8
Ser	10	12
Glu	8	10
Pro	6	7
Gly	16	17
Ala	4	6
Val	9	8
Met[a]	2	2
Ile	7	7
Leu	7	8
Tyr	6	6
Phe	7	7
Trp	ND[b]	ND
Total	110	119

Average results from ten analyses. Data from reference 11, with permission of Pergamon Press.
[a]Determined as methionine sulfone.
[b]ND: not determined.

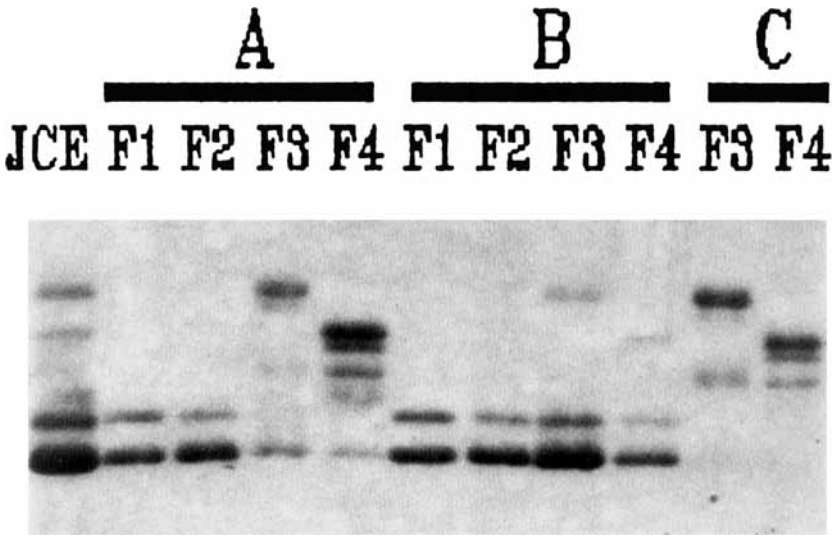


Fig. 1. 15% SDS-PAGE analysis of JCE and DEAE fractions F1 to F4. **A:** Whole fractions. **B:** Their IgA-binding subfractions. **C:** Non-IgA-binding subfractions from fraction F3 and F4 (corresponding subfractions from F1 and F2 contained no detectable material). Coomassie blue staining, unreduced gel.

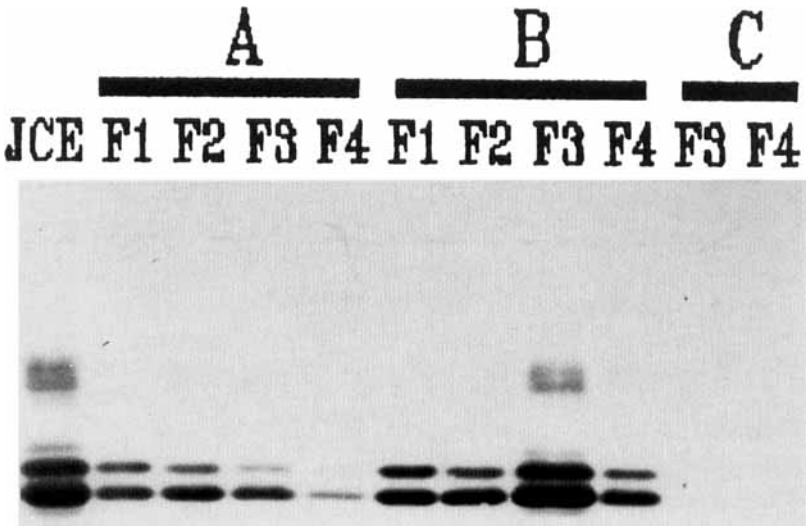


Fig. 2. Same gel as in Figure 1, Western blot with peroxidase-coupled F(ab')2 fragments of antijacalin antibodies.

ilar to that in F2; the proportion of unglycosylated subunits in fraction F4 is higher (ratio: 9.5) (13). In JCE as well as in jacalin purified by affinity chromatography from JCE, this ratio is 3, which first led us to conclude, in view of gel filtration studies of the native molecules, that jacalin was made up of one glycosylated and three unglycosylated subunits (11). In fact, as already mentioned, it presents a striking heterogeneity of electric charge, the reason for which remains unclear, but it could be related to the presence of molecules differing by the proportion of both subunits. When electrophoretic analysis is performed at low pH (4.3 or 4.5), this heterogeneity of charge is no longer observed (12,14,15); this phenomenon probably is due to the dissociation of the subunits, which probably also explains why the lectin loses its reactivity below pH 4.5 (16). In addition, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of fraction F3, under both reducing and nonreducing conditions, shows molecules of higher Mr than the subunits (the major one has an apparent Mr of 26 kD under reducing conditions) that bind IgA and react with antijacalin antibodies (Figs. 1, 2). It is also worth mentioning that fraction F4 contains several non-IgA binding molecules that are unreactive with the antijacalin antibodies. Some of these molecules yield two components of the same size as the jacalin subunits upon reduction.

Although most recent studies confirm the above-mentioned data characterizing jacalin, a few discordances are worth noting. Kumar et al. (14) reported that the lectin is made up of four identical 10.5 kD subunits. We found the N-terminal amino acid to be glycin, whereas Basu et al. (17) found a methionine. These minor discrepancies might be related to apparent differences of reactivity of crude extracts from jackfruit seeds of different geographical origins (8,18) (see below).

CARBOHYDRATE SPECIFICITY

Jacalin is known to be an α D-galactoside-specific lectin (14). The use of p-nitrophenyl-α D-galactoside and 4-methylumbelliferone-α D-galactoside allowed evaluation by equilibrium dialysis and fluorescence quenching of its association constants with these ligands (Ka = 1.4×10^5 and 2.1×10^5 M^{-1} at 25°C), which led to the conclusion that there are only two binding sites per molecule (19). This conclusion, although based on the hypothesis of a homogeneous and homotetrameric structure of the lectin (14), which has not as yet been confirmed by others (see above), is worth noting because it would represent the first case of a lectin whose binding activity requires the quaternary association of subunits. Appukuttan and Basu (20) also demonstrated the importance of lysyl and tyrosyl residues to binding activity. The involvement of tyrosyl explains at least in part the intrinsic fluorescence changes of the lectin after ligand binding. This property allowed demonstration that C1, C2, C4, and C6 galactose hydroxyl groups are determinant (21); inversion of C4 hydroxyl in the glucose molecule and absence of C6 hydroxyl in fucose thus explain the nonreactivity of these sugars. On the other hand, the α-anomer of galactose binds more strongly than does the β-anomer.

Thermodynamic studies of jacalin binding to several monosaccharides and disaccharides showed the strongest ligand by far to be DGalβ1→3DGalNAc (22). In agreement with this, we independently found that N-acetylgalactosamine and galactose are the two monosaccharides that are the strongest inhibitors of binding of jacalin to IgA coats, with lactose showing a much lower but significant inhibition capacity (11) (Fig. 3). This latter result does not confirm a previous work (16) in which lactose was found to be unreactive, but it agrees

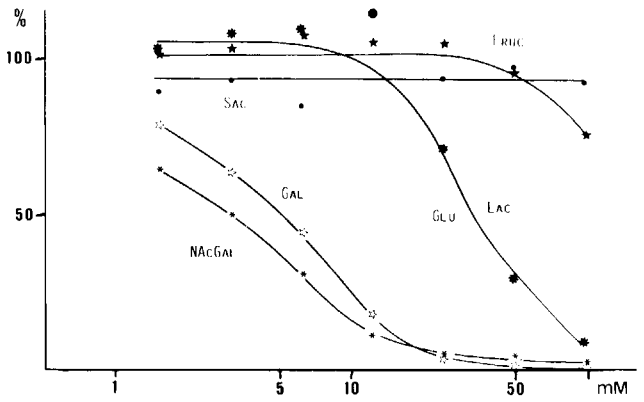


Fig. 3. Competitive ELISA: inhibition of binding of jacalin-peroxidase to IgA1 coats by increasing concentrations of N-acetylgalactosamine (NAcGal), galactose (Gal), glucose (Glu), lactose (Lac), saccharose (Sac), and fructose (Fruc).

with similar findings by other investigators (22). The β1→4 linked glucose and N-acetylglucosamine C3 hydroxyl groups present in lactose and N-acetyllactosamin, respectively, and N-acetylglucosamin C4 hydroxyl in Galβ1→3GlcNAc explain the much lower affinity of these ligands. Galβ1→3GalNAc residues are o-glycosidically linked to serine or threonine in numerous animal proteins. Consequently, jacalin can precipitate a variety of proteins, such as ant egg glycoprotein and human glycophorin (23). Also, a precipitation reaction was observed between jacalin and certain batches of bovine serum albumin, suggesting that this reaction was due to contaminants. Finally, jacalin agglutinates human erythrocytes of all blood groups (10,14); it binds to the surface of all human peripheral blood cells, as shown by direct immunofluorescence with fluorescein-conjugated jacalin (unpublished results).

REACTIVITY WITH IMMUNOGLOBULINS

Precipitating Assays

In most studies, including the present one, of the reactivity of jacalin with human Ig, only IgA1 and IgD were found to be precipitated by jacalin (7,9,11,24); this specificity, which was also clear when Western blot methods were used (11), is well explained by the presence of o-glycosidically linked Galβ1→3GalNAc (ocassionally sialylated) residues on the hinge region of α1 and δ chains (25,26) but not on other human Ig heavy chains. Using proteolytic fragments yielded by *Neisseria gonorrhea* and *Streptococcus sanguis* proteases, Skea et al. (27) confirmed that jacalin reacts with the hinge region of IgA1. In precipitation assays, the subclass specificity is clear, as all 76 monoclonal IgA1 and none of the seven IgA2 [5 IgA2m(1) and two IgA2m(2)] tested were precipitated by jacalin. This provides a simple and reliable method for typing myeloma IgA (28). IgD reactivity is more controversial; whereas all 13 myeloma IgD assayed in our laboratory and six more tested by others (24) were precipitated by jacalin, some authors found this isotype to react only after neuraminidase treatment (15) or not at all (7). It is worth recalling that IgD is very prone to proteolysis and that its degradation quickly impairs its jacalin reactivity, even while it is still precipitated by anti- δ antisera (11), which might explain these inconsistencies. The lectin does not precipitate IgG, IgM, and IgE according to most studies.

In contrast, although they observed a similar specificity with JCE from seeds collected in the Philippines, Kondoh et al. (8) found positive reactions by gel double-diffusion between a half-purified jackfruit seed preparation from Okinawa and IgM, IgA2m(2), and IgE (in addition to precipitation with IgA1 and IgD); later on, these same authors reported the reactivity of IgA2 of both allotypes, IgM, IgE, and even aggregated IgG with a preparation from a peculiar batch of jackfruit seeds that were "rounder and thicker in shape" than others (18). It was suggested that these differences might reflect variations of the properties of jacalin according to the geographic origin of the jackfruit seeds. Despite the great potential interest of these results, they probably should be viewed cautiously because of some methodological points, which have been previously discussed (9). Jacalin from seeds originating from three distinct geographic areas—Brazil, La Reunion, and the Philippines—were used in the present study, and no differences were found; however, this does not rule out the possibility of geographical and even species-related differences in jacalin preparations. There are many species of *Artocarpus*, each with a number of vernacular names and scientific synonyms. This results in some confusion in immunologic papers; for example, *A. integrifolia* and *A. integra* sometimes were considered the same, sometimes as different (29) species. It is, however, clear from botanical publications (30–33) that the jack (*A. heterophyllus*, most common synonyms *A. integrifolia* and *A. integra*), which originates from India and has spread throughout the tropics since prehistory has well-known botanical characteristics that allow an easy distinction with other trees of the same genus (also cultivated for their edible fruits), such as the breadfruit (*A. altilis*) and the champedak (*A. integer*). However, there is a great variability within the single species *A. heterophyllus*. Perhaps more importantly, natural or artificial hybridization between *A. heterophyllus* and *A. integer* is known to occur, for example, in the Philippines.

Nonprecipitating Assays

Analysis of Ig-jacalin interactions by nonprecipitating assays is a matter of controversy, especially concerning IgA2. This is a critical issue, as jacalin, which can easily be coupled to Sepharose beads by the cyanogen bromide method, is undoubtedly a very efficient tool to purify IgA and IgD or to remove IgA from therapeutic IgG preparations, for instance (11). Jacalin thus makes it much easier to obtain pure IgA1 or IgA2 from myeloma sera. IgA2 was not found in material purified

from a few sera by jacalin-affinity chromatography (24,27), but it was evaluated in one of these studies (27) by a rather insensitive assay (protease resistance). In another study (15), inhibition by IgA2 of the agglutination by jacalin of latex beads coupled to IgA1 was assumed to reflect contamination by IgA1. In contrast, we found a reactivity of IgA2 with jacalin that was much weaker than that of IgA1 but significant nonetheless (11), and attempts to separate IgA subclasses by jacalin-agarose affinity chromatography were not always completely successful. Thus, Gregory et al. (34) obtained pure IgA2 by passing colostrum on a jacalin column, but the eluted IgA1 were contaminated by 8% IgA2. On the other hand, the procedure described by Kondoh et al. (8) to purify secretory IgA1 and IgA2 does not exclude, in our opinion (9), the possibility of a contamination of the IgA1 fraction by IgA2 because of the lack of sensitivity of control experiments. We recently confirmed that IgA2 of both allotypes binds to jacalin agarose. However, monoclonal IgA2 can hardly be purified from myeloma sera in this way, because jacalin-binding proteins, although they contain fair amounts of IgA2, are considerably enriched in residual IgA1 (11,35) (Fig. 4). Indeed, inhibition experiments of the binding of jacalin to IgA1-coated polystyrene by human Ig of each isotype show that IgD and IgA1 display a much higher apparent affinity than does IgA2 (35) (Fig. 5). It is worth noting that, in our hands, IgA2 shows a very similar inhibition of binding of IgA1 to jacalins from Brazil, La Réunion, and the Philippines. This affinity difference allows the removal of IgA1 from IgA2 preparations. In unpublished experiments, four IgA2 myeloma preparations and a normal serum pool were incubated for 1 hour at 25°C and for 2 hours at 4°C, with increasing amounts of jacalin or JCE coupled to cyanogen bromide-activated Sepharose; IgA1 and IgA2 levels then were measured by radial immunodiffusion. These experiments allowed estimation of the amount of immobilized jacalin required to eliminate completely 1 mg of IgA1, with a minimum loss of IgA2, of 2 mg. On the other hand, a consequence of the reactivity of jacalin with IgA belonging to both subclasses is its possible use to remove readily and completely IgA from IgG preparations for therapeutic use (11), which may be critical when dealing with patients presenting with anti-IgA antibodies or known to be prone to the development of such antibodies, as in combined IgA and IgG2 deficiency.

Conversely, IgA1-affinity chromatography provides an easy way to purify the lectin from JCE (11–13,15,27,36). Because of its reactivity with galactomannans (16), insolubilized guar gum can also be used to prepare jacalin, which is possibly a simpler method (37). However, it should be pointed out that preparative methods based on protein charge, such as DEAE chromatography or zone electrophoresis on Pevikon C870, yield pure jacalin (according to SDS-PAGE with silver staining and demonstration of a single N-terminal amino acid) with a remarkable yield (11).

Jacalin does not bind mouse, rat, pig, goat, horse, cow, or dog IgA (38,39). We are not aware of any data concerning its reactivity with rabbit serum Ig; preliminary results from our laboratory might indicate that jacalin could bind rabbit IgG and their pepsin F(ab′)2 fragments (unpublished), which should be related to a peculiar glycosylation of the hinge region of some of these molecules (40). Finally, rabbit secretory IgA binds jacalin (38); this property might be useful for the study of the glycosylation of this Ig class, which displays an important isotypic diversity in this species (41).

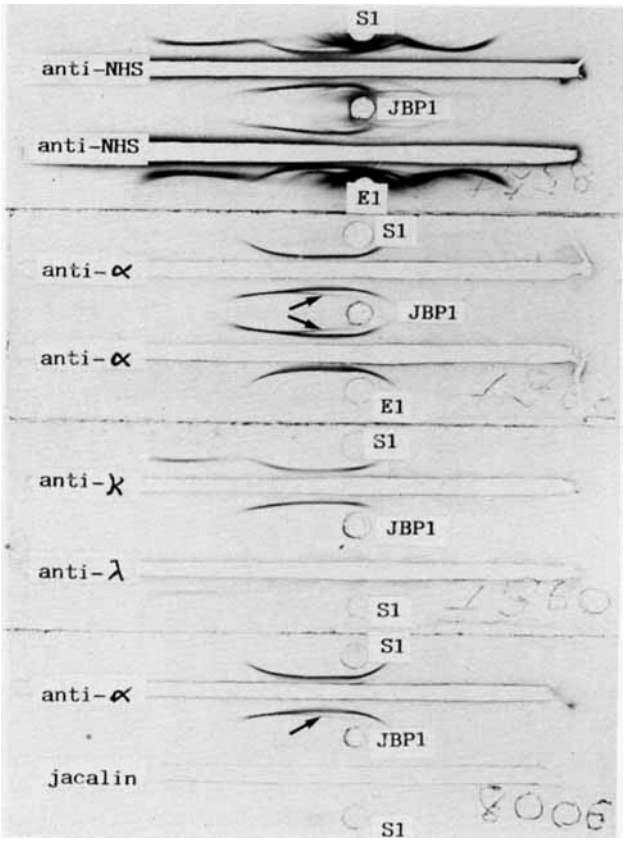


Fig. 4. Immunoelectrophoretic analysis of jacalin-binding proteins (JBP1) and effluent (E1) obtained by affinity chromatography of an ammonium sulfate precipitate from an IgA2m(1)x myeloma serum (S1). Anti-NHS: polyvalent antinormal human serum antiserum. Note (1) that, although the bulk of the IgA2 is in the effluent, it is also present in JBP, and (2) the spur of polyclonal IgA1 over IgA2 (arrow). Data from reference 35, with permission of Elsevier.

MITOGENIC PROPERTIES

The finding that JCE is mitogenic for human peripheral blood mononuclear cells (PMBC) (3,4) led us to investigate the modalities of this activity. All four DEAE fractions (see above) display such an activity; however, as demonstrated by adsorption on human IgA1, reactivity with antijacalin antibodies on Western blots and study of the inhibition of lym-

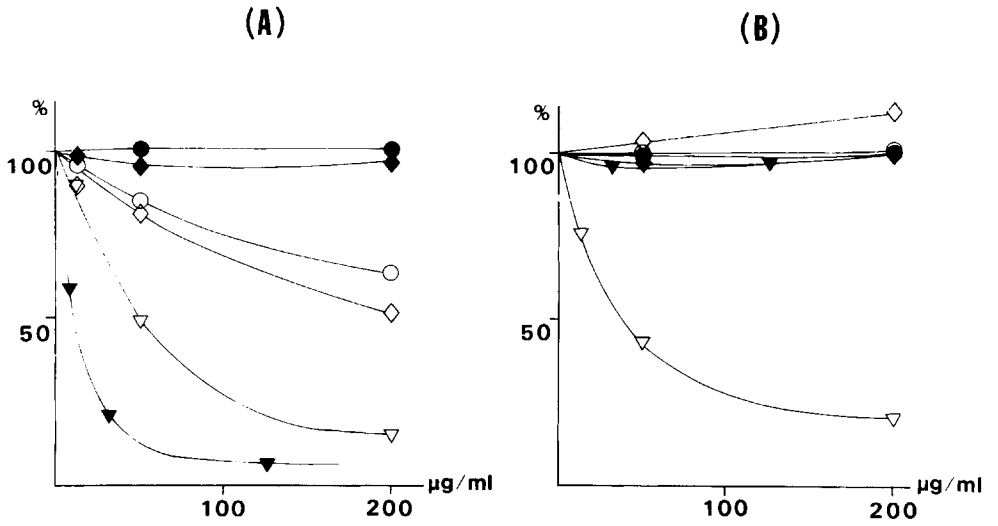


Fig. 5. Inhibition of binding to IgA1 coats of jacalin (**A**) and of the anti-α1 monoclonal antibody M4D8 (**B**) by purified IgA1x (▽), IgA2m(1)x (○), IgA2m(2)x (◇), IgDλ (▼), IgG3x (●), and IgMx (◆). Percentages of optical densities recorded in the absence of inhibitor.

phocyte stimulation by IgA1, the activity of fractions F1, F2, and F4 is entirely due to jacalin, whereas F3 also contains one or more other mitogenic molecule(s) (13).

When incubated with peripheral blood mononuclear cells (PBMC) at the optimum concentration of 200 µg/ml, jacalin leads to a maximum ^{3}H-thymidine incorportion on day 3 of culture. Although it can bind virtually all blood cells, in our hands, only T lymphocytes were stimulated. In addition, jacalin fails to induce any plasma cell differentiation of blood B cells, as shown by cytoplasmic immunofluorescence with anti-Ig conjugates. This observation does not confirm the first study (3), but it is in agreement with more recent data (4). We have studied the mitogenic properties of jacalin with respect to T-cell subpopulations (13). Indirect immunofluorescence studies of membrane antigens with monoclonal antibodies showed that after 7 days of culture of PBMC with jacalin, CD4-bearing T cells accounted for most viable cells. Monocyte depletion of PBMC abolishes almost completely the mitogenic activity of jacalin. Similarly, isolated CD4 and CD8 T cells fail to respond to jacalin. The proliferative response of purified CD4-positive cells (and not that of CD8 cells) is recovered by the addition of syngeneic (but not allogeneic) monocytes or partially by recombinant interleukin 1 (Fig. 6). Monocytes primed by 24-hour culture with jacalin and then washed are unable to stimulate lymphocytes. In fact, short incubations (1–18 hours) of unseparated PBMC with jacalin followed by washing and further culture for up to 4 days fail to induce any proliferative response.

A recently published study by Gattass et al. (5) reported that jacalin could inhibit OKT8 antibody binding to PBMC. However, cocapping experiments using fluorescein-conjugated jacalin and anti-CD1, CD2, CD3, CD4, CD8, and CD38 antibodies performed in our laboratory did not show any significant binding of the lectin to these antigens. It is

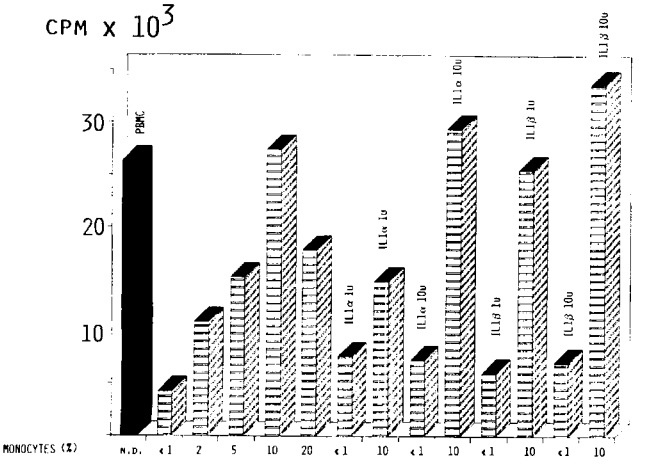


Fig. 6. Proliferative response of normal human PBMC and purified CD4 T cells in the presence of increasing percentages of monocytes and of IL1 (data from eight experiments).

worth noting that JCE induces the production of γ-interferon by human T cells (42).

PERSPECTIVES

As discussed above, the structure and properties of jacalin are already well documented, and there is every reason to believe that its immunological applications will be increasingly utilized. It is worth mentioning that other lectins can react with Ig. Among them, PHA displays an interesting isotypic specificity, as it precipitates only IgA2, pentameric IgM, and some polymeric IgA1 (43). Therefore, together with jacalin, PHA may be used to characterize monoclonal Ig in lymphoproliferative disorders (44,45). Lymphocyte polyclonal activation and serum protein precipitation activities of PHA are due to different entities (46,47). On the contrary, jacalin

is a well-defined molecule and both its effect on lymphocytes and its reactivity with Ig are related to the same well-characterized lectinic property. Although, as already discussed, jacalin allows the distinguishing of human IgA subclasses by gel precipitation tests, it must be emphasized again that it also binds IgA2 of both allotypes in nonprecipitating assays. Therefore, its use for separating IgA subclasses is not in itself a purity criterion, and technical conditions must be adapted to take advantage of the difference in the affinity of IgA1 and IgA2.

PHA and jacalin have also some homologies with respect to their mitogenic properties, as they both stimulate T cells. However, although PHA induces the proliferation of all T-cell subsets (48), jacalin acts only on CD4-positive cells. In addition, the presence of monocytes is required for jacalin stimulation, whereas PHA can recruit other accessory cells, including lymphocytes (49). The CD4 cell specificity of jacalin makes it a potential tool for a functional study of these cells, especially in immunodeficiency states in which this lymphocyte subset is impaired. Our preliminary results in HIV-infected patients (50) showed that the in vitro response to jacalin is indeed depressed, with a significant correlation with the number of blood CD4 lymphocytes. In certain cases, response to other mitogens such as PHA remains normal, whereas jacalin-induced proliferation is strongly reduced. The question remains open as to whether this abnormality is due to monocyte or lymphocyte functional defect and whether it has any prognostic significance.

In addition to these major applications, the use of jacalin has been considered for preparing certain polysaccharides (51) or glycoproteins. Thus, Hiemstra et al. (36) reported that complement C1 inactivator can be purified by jacalin affinity chromatography without losing its activity; moreover, these authors demonstrated that jacalin activates the classical pathway of complement by blocking C1 inactivator. The use of jacalin thus may be envisaged in the exploration of any biological system in which it could interfere with regulatory glycoproteins.

ACKNOWLEDGMENTS

This work was supported in part by INSERM-CNRS Programmes National de Recherche sur le SIDA, and by Fondation pour la Recherche Médicale.

The authors thank Professors J. Barrau (Museum National d'Histoire Naturelle, Paris) and J.L. Pousset (Poitiers) for helpfull discussions and Dr. P. Goube de Laforest (Poitiers) for his help in the study.

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