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The Potent IgG4-Inducing Antigen in Banana Is a Mannose-Binding Lectin, *BanLec-I*

Key Words

IgG4
Banana
Lectin

Abstract

IgG4 antibodies to banana were found to occur far more frequently than expected. The most important antigen involved proved to be a lectin, *BanLec-I*. Because of the lectin nature of the antigen, it was important to establish the antibody nature of the lectin-IgG4 interaction and to exclude an interaction between the sugar-binding site of the lectin and glycosidic chains on IgG4. Three arguments in support of immune binding are: (1) the binding of *BanLec-I* to IgG4 is mannoside resistant, whereas the binding to all other glycoproteins tested is mannoside inhibitable; (2) only a minor fraction of the IgG4 in serum and none of five IgG4 myelomas tested was bound, and (3) the lectin binds to the Fab fragment of the IgG4 molecule. A curious finding was that in the presence of high-molecular-weight glycoproteins the interaction between IgG4 and *BanLec-I* was enhanced by α -methyl mannoside. The probable explanation of this phenomenon is that complexes of the lectin with high-molecular-weight glycoproteins by steric interference inhibit the interaction with human IgG4 antibodies (or with rabbit antibodies to the lectin). This inhibition is prevented in the presence of α -methyl mannoside. These results support the earlier suggestion that some lectins are particularly prone to induce an immune response upon oral feeding. This banana lectin might be a potentially useful carrier protein for oral antihapten immunization in humans.

Introduction

The human IgG4 immune response is characterized by a slow onset relative to other isotypes. A strong IgG4 response requires prolonged immunization [1]. This finding suggests that the stimulation requirements for B cells committed to this isotype are distinct from those of the other isotypes, e.g., IgG1.

During an investigation of the antibody response to a broad panel of ingested foods [2, 3], we found that the IgG

antibody response to banana extract was much stronger than expected on the basis of quantities commonly ingested. This was particularly the case for IgG4 antibodies and much less so for IgG1 or IgA antibodies. Furthermore, the antibody titer did not drop above the age of 10, as the antibody titers to other foods tend to do. Therefore, we considered it of interest to investigate the antigen responsible for this pronounced IgG4 response. During this investigation, we found that the IgG4-binding activity was due to the presence in banana of a lectin with α -methyl-D-

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mannopyranoside (mannoside) specificity [4]. We proposed its name to be *BanLec-I*.

This lectin activity necessitated further experiments to distinguish possible nonimmune binding of the lectin to carbohydrates on the IgG4 molecule from the immune binding of IgG4 antibodies to the lectin molecule. Therefore, we tested the effect of lectin-binding sugars on the binding of IgG4 to the lectin. A puzzling finding was that low-molecular-weight sugars (e.g., mannoside) were not inhibitory, whereas high-molecular-weight glycoproteins were. A possible explanation for this inhibitory effect of glycoproteins was offered by the paradoxical effect of mannoside on the inhibition by glycoproteins. Mannoside proved to counteract the inhibition of the binding of the lectin to IgG4 by high-molecular-weight glycoproteins. This positive effect of mannoside was further investigated, particularly because this effect was also found with antibodies induced by immunization of rabbits with the lectin.

Materials and Methods

Banana lectin was isolated from banana extract by affinity chromatography on Sephadex G-75 with 0.1 M mannoside as eluting agent [4]. Egg, milk, soybean, peanut, rice, crab, and oyster extracts were prepared by extracting 10% uncooked material (w/v) in water at pH 8.0 for 2 h and centrifuged. The supernatant was dialyzed against water and then lyophilized. Banana and potato extract were prepared in a phosphate buffer containing polyvinylpyrrolidone [5]. A rabbit-antibanana antiserum was prepared by immunization with a crude *BanLec-I* fraction, prepared by hydrophobic chromatography of banana extract on phenyl Sepharose [4]. Monoclonal antihuman IgG4 (MH 164-1-MA2) was obtained from the Department for the Production of Immune Reagents in our Institute.

Human Antibodies

NHS-1 was a pooled human serum, purchased from Behringwerke (Marburg, FRG). NHS-2 was the serum pool of healthy military blood donors. NHS-3 was the defibrinated blood plasma pool which was obtained from normal blood donors. The two serum samples used in the absorption experiments, catpositive and mitepositive, were obtained from the Allergy Diagnostic Laboratory of our Institute. One patient was receiving immunotherapy with catdander extract and another with miteculture extract. A group of sera were collected from children under the age of 7 years with suspected atopy. These children participated in the study performed by Calkhoven et al. [3]. The sera were obtained after informed consent of the parents. For most subjects, no information was available on the dietary history with respect to banana. However, for 1 subject we tested the effect of banana avoidance for 4 weeks, followed by a challenge with five bananas. No change in titer was observed. From this group, 40 sera that had IgG4 antibodies to banana were used for the comparison of two IgG4-binding assays and two *BanLec-I*-binding assays. The ten serum samples used for the inhibition experiments were selected from the Allergy Diagnostic Laboratory of our Institute. They all had IgG4 antibodies to banana. Pooled normal human IgG (160 mg/ml) was ob-

tained from our Institute. Five purified IgG4 myeloma proteins were kindly provided by Dr. A. Vlug of the Department of Immune Reagents of our Institute.

Sepharose-Coupled Reagents

Sepharose-coupled protein A was purchased from Pharmacia (Uppsala, Sweden). All other extracts (5 mg each) were coupled to 100 mg CNBr-activated Sepharose (Pharmacia), according to the manufacturer's instructions, with the exception that after coupling the Sepharose was inactivated by 0.5 M glycine. A glycine-coupled Sepharose was also prepared the same way to be used as a control Sepharose. Banana Sepharose was prepared by coupling 5 mg of dialyzed banana extract (prepared in a buffer containing polyvinylpyrrolidone) to 100 mg activated Sepharose. Anti-IgG4 Sepharose was prepared by coupling 4 mg of purified monoclonal antibody to 400 mg activated Sepharose.

Iodinated Reagents

BanLec-I was iodinated as previously described [4]. Anti-IgG4 (75 µg) was iodinated with 1 mCi of carrier-free ¹²⁵I (Radiochemical Centre, Amersham, UK) by the chloramine T method, and the excess of unbound ¹²⁵I was removed by gel filtration on an ACA-44 column (LKB, Bromma, Sweden).

IgG4 Antibodies to Various Food Antigens

The three NHS pools were tested for IgG4 antibodies to various food antigens coupled to CNBr-activated Sepharose 4B. The food-antigen-coupled Sepharose (0.5 mg) in 500 µl of phosphate-buffered saline/0.05% Tween 20/0.190 human serum albumin (PBS-AT) was incubated with 20 µl of NHS at a dilution of 1:9. After an overnight incubation, the Sepharose was washed five times, resuspended in 500 µl of PBS-AT, and incubated overnight with iodinated monoclonal anti-IgG4. The Sepharose was washed again four times, and the bound anti-IgG4 was expressed as the percentage of added radioactivity. The nonspecific binding of the anti-IgG4 to the Sepharose in the absence of the serum was less than 2%. When purified IgG4 myelomas were tested with the banana Sepharose in a similar way, the binding of anti-IgG4 was less than 4%.

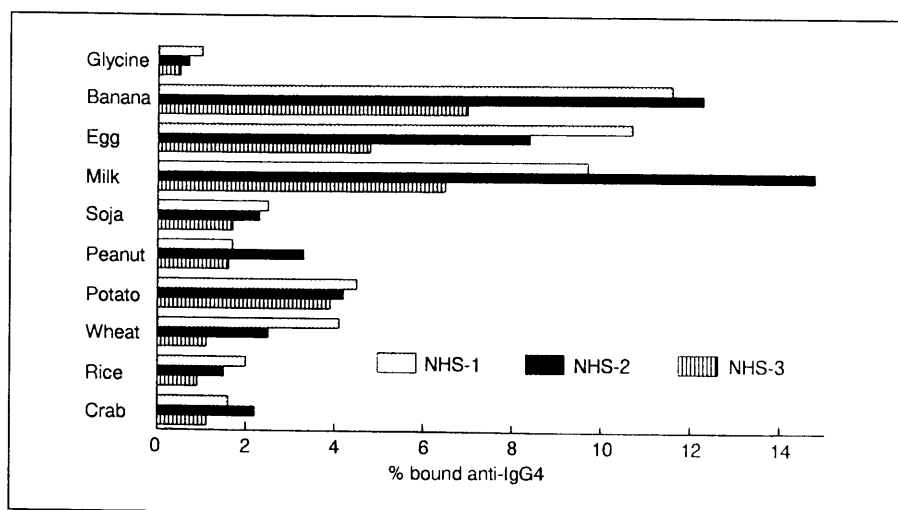
Absorption of Serum on Sepharose-Coupled Banana Extract

The two sera, cat-positive and mite-positive serum with a high IgG4 binding to banana Sepharose, were selected from sera of patients who received immunotherapy with cat dander extract and mite culture extract, respectively; the first had a high titer of IgG4 antibodies to cat allergens, the other to mite allergens. Each serum sample (100 µl) was incubated overnight with 100 mg banana Sepharose (equivalent to 5 mg dialyzed lyophilized banana extract) in a final volume of 500 µl PBS-AT. The activity of IgG4 antibodies to the respective allergens remaining in the supernatant was measured using a radioallergosorbent test (RAST).

Papain Fragmentation of Human IgG

Pooled normal human IgG, 25 mg in 150 µl of PBS, was treated with 0.25 mg papain (Merck 3.5 m Anson-E/mg, 2.5 mg dithiothreitol, and 0.37 mg disodium ethylenediaminetetraacetate (EDTA) in a total volume of 500 µl PBS. The pH of this solution was adjusted to 5.5, and the solution was incubated for 18 h at room temperature. At the end of the reaction, 6.25 mg of N-ethyl maleimide was added, the pH of the solution was adjusted to 7.4, and the solution left at room temperature for another hour. This solution was then dialyzed against

Fig. 1. NHS-1, NHS-2, and NHS-3 were tested for IgG4 antibodies to banana (in the presence of mannoside), egg, milk, soya, peanut, potato, wheat, rice, and crab coupled to CNBr-inactivated Sepharose 4B. Glycine-coupled Sepharose was used as a negative control. The results are shown as percentage of bound counts of iodinated anti-IgG4.



PBS containing 0.5 M NaCl for 48h. After dialysis, the residual intact human IgG was removed by gel filtration on a column (15 x 400 mm) of Sephacryl 300 (Pharmacia). A fraction containing both Fc and Fab was electrophoresed in 1.3% agar in 10 mM sodium barbitone buffer (pH 8.6) for 55 min. Upon completion of the electrophoresis, the trough was filled with rabbit antiserum (obtained in our Institute) to either total IgG, anti-Fc, or anti- κ light. The slides were washed for 2 days with PBS and equilibrated overnight with PBS containing 0.5 M mannoside. Then 150 μ l of labeled (50,000 cpm) *BanLec-I* in 0.5 M mannoside was put in the trough and allowed to diffuse overnight. The slides were again washed with PBS for 2 days, dried, and exposed for 48h to Kodak X-ray film with an intensifying screen (Du Pont USA) at -30°C.

Antibody Assays

Human IgG4 antibodies were measured either by testing sera with Sepharose antigen and detection of bound IgG4 with iodinated monoclonal anti-IgG4 or by incubating sera with Sepharose-coupled monoclonal anti-IgG4 and detecting bound antibody with iodinated, purified *BanLec-I*. Unless indicated otherwise, the incubations were performed in the presence of 100 mM mannoside. Rabbit or human IgG antibodies were similarly measured using Sepharose-coupled protein A and iodinated *BanLec-I*.

Inhibition of Banana IgG4 RAST by BanLec-I

The ten serum samples containing IgG4 antibodies to banana were randomly selected. NHS-2 was also used for this purpose. Serum (2.5 μ l) was preincubated for 3 h with 5 μ g of *BanLec-I* in 100 μ l PBS-AT containing 0.2 M mannoside. This mixture was then incubated overnight with 250 μ l banana Sepharose (1 mg/ml). Then, Sepharose-bound IgG4 was measured with iodinated anti-IgG4.

Inhibitory Effect of High-Molecular-Weight Glycoprotein

The above-mentioned sera were used in this assay. Serum (0.25 μ l) was incubated with 0.2 mg protein A-Sepharose in 250 μ l PBS-AT. The Sepharose was washed and suspended in 500 μ l of PBS-AT containing 50 μ l crab, soya, or oyster extract (2 mg/ml) in the presence or absence of 100 μ l of 1 M mannoside and 50 μ l iodinated *BanLec-I*. After an overnight incubation, the Sepharose-bound activity was measured.

Agar Electrophoresis

Agar electrophoresis of the serum proteins was performed by electrophoresing 2.5 μ l of a serum sample in 0.9% agar for 55 min. Five sera each with a fresh and aged serum sample were electrophoresed. Upon completion of the electrophoresis, the separated serum proteins were passively blotted on a nitrocellulose sheet. The nitrocellulose sheet was washed three times with PBS containing 0.01% Tween and 0.01% human serum albumin for 30 min each time. It was then incubated overnight (400 x 10³ counts/min) with ¹²⁵I-*BanLec-I* in the same buffer. The next day, the nitrocellulose sheet was washed five times with PBS containing 0.01% Tween, air-dried, and exposed to a Kodak X-ray film for 40 h with an intensifying screen. After developing the film, the nitrocellulose sheet was incubated overnight with 50 ml of 0.5 M mannoside, followed by washing, drying, exposing, and developing as above.

Results

Previously it was found that the median titer of IgG4 binding to banana was strikingly high as compared with other foods; the IgG1 binding was not particularly high. To emphasize this point, we tested the IgG4 antibodies to diverse food antigens in three NHS pools of different origins. Figure 1 shows that all three NHS pools contain high titers of IgG4 antibodies to the banana.

Specificity of the Binding

When we attempted to fractionate a partially purified banana extract on a Sephadex G-75 column, we noticed that the IgG4-binding activity was lost; the activity was recovered upon elution with mannoside. This clearly suggested that a mannose-binding lectin was involved. The important question was, therefore, whether the binding of IgG4 to the lectin was due to a binding of the lectin to the carbohydrates on IgG4 or, alternatively, due to binding of

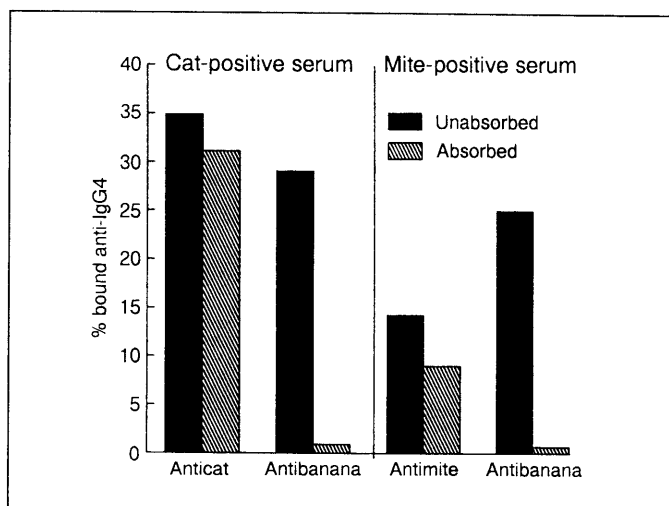


Fig. 2. Absorption of two sera with Sepharose-coupled banana extract. One serum had IgG4 antibodies to cat and another to mite. Absorption removed all the IgG4 antibodies to banana but not to their respective antigen. A slight loss of anticat and antimite titer is due to the nonimmune binding of the glycosidic chain of the IgG4 molecule to the *BanLec-I* in the banana extract.

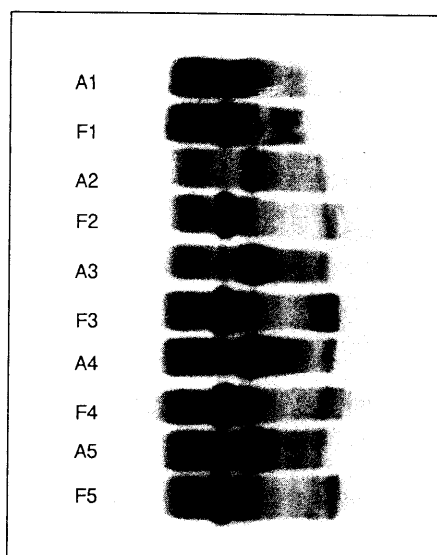


Fig. 3. Binding of ^{125}I -labelled *BanLec-I* to serum proteins. Proteins were separated by agar electrophoresis and transferred to nitrocellulose. The anode is on the right. Serum samples of donors 1–5 were tested fresh (F) and aged (A). Note the strong staining of complement factor C3.

the lectin to the antigen-binding site of IgG4 antilectin antibodies.

To establish the specificity of the IgG4 binding to banana, we performed solid-phase absorption experiments

with Sepharose-coupled banana extract. These studies indicated a high degree of specificity. After absorptions, the antibanana activity of IgG4 antibodies was almost completely removed, whereas there was only a small decrease of the IgG4 antibody titer to the cat allergens or to the house dust mite allergens (fig. 2). Moreover, the purified IgG4 myeloma proteins did not bind to banana Sepharose.

Detection of IgG Antibodies and Serum Proteins

To detect the binding nature of *BanLec-I* to the IgG antibody, the nitrocellulose blot of agar-electrophoresed serum samples was incubated with radiolabeled *BanLec-I*. ^{125}I -*BanLec-I* detected various serum glycoproteins, e.g., the gamma globulin fraction; the strong reactivity with both the native and converted C3 in fresh and aged serum samples, respectively, was remarkable. No reaction with transferrin was found, and, as expected, the lectin did not bind to the albumin (fig. 3). However, on incubation of this nitrocellulose blot with mannoside, the carbohydrate-binding activity of the *BanLec-I* to all the serum glycoproteins was removed as a result of nonimmune binding, except in the case of the IgG fraction which retained its binding as a function of the classical antigen-antibody complex.

Papain Fragmentation of IgG

A fraction containing papain-treated human IgG without intact IgG was electrophoresed in agar and precipitated with antibodies to human IgG and anti-IgG (Fc and Fab). Binding of radiolabeled *BanLec-I* in the presence of mannoside was found only to the Fab Fragment; no binding to Fc was detected (fig. 4).

Contribution of IgG4 to the Total IgG BanLec-I-Binding Activity in Serum

A group of 40 sera obtained from children under the age of 7 years, selected on the basis of a positive banana IgG4 RAST in the absence of mannoside, were incubated either with protein A-Sepharose or with Sepharose-coupled anti-IgG4 and subsequently with radiolabeled *BanLec-I* in the presence of 0.1 M mannoside. The results shown in figure 5 clearly indicate that the response in most sera is largely due to IgG4.

Contribution of BanLec-I to Total Banana IgG4 Antibody-Binding Activity

A group of the 40 sera mentioned above was tested in two assay systems. In one assay, the sera were incubated with total banana extract coupled to Sepharose; bound IgG4 was measured by radiolabeled anti-IgG4. In the other assay, sera were incubated with Sepharose-coupled

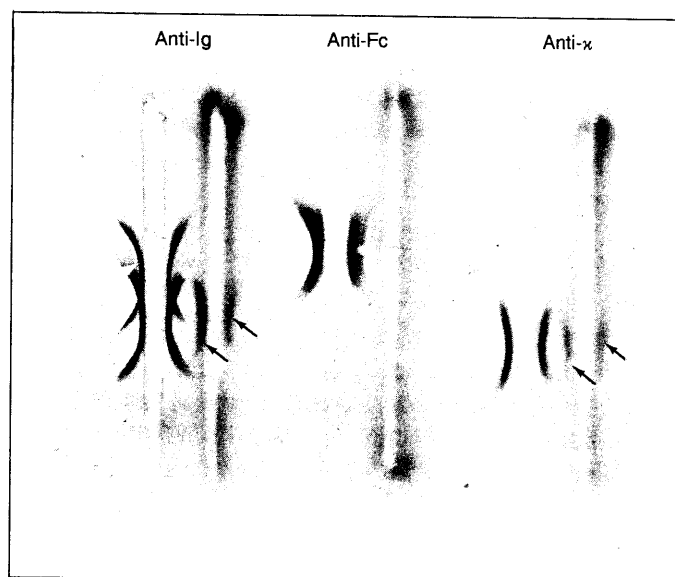


Fig. 4. Immunoelectrophoresis of human Fab and Fc fragments with rabbit anti-Ig, anti-Fc, and anti- κ . Adjacent to each of the stained precipitin arcs is the autoradiography of the precipitin arc (arrows) obtained after reaction with radiolabeled *BanLec-I*.

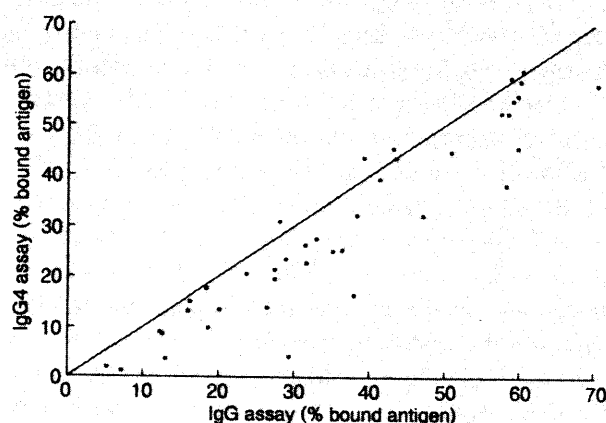


Fig. 5. Comparison of two *BanLec-I*-binding assays. On the x-axis, serum was incubated with protein A-Sepharose and the bound IgG measured by radiolabeled *BanLec-I*. On the y-axis, serum was incubated with Sepharose-coupled monoclonal anti-IgG4 and the bound IgG4 measured by radiolabeled *BanLec-I*.

monoclonal anti-IgG4, and bound IgG4 was measured by radiolabeled, purified *BanLec-I*. Both assays were performed in the presence of 0.1 M mannoside. A good correlation was found between these two assays (fig. 6). A few sera, however, reacted to the total banana extract and not

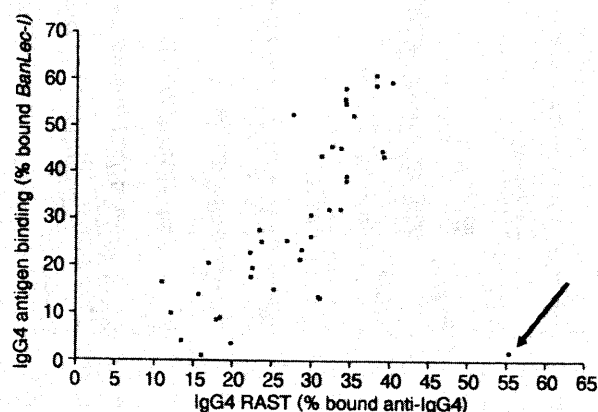


Fig. 6. Comparison of the IgG4 assays. On the abscissa, the IgG4 response is plotted towards total banana extract measured with the IgG4 RAST: serum was incubated with banana Sepharose and bound IgG4 measured with radiolabeled anti-IgG4. On the ordinate, the results are plotted of the IgG4 response towards purified *BanLec-I*: serum was incubated with Sepharose-coupled anti-IgG4 and the bound IgG4 measured with radiolabeled *BanLec-I*. Both assays were performed in the presence of 0.1 M mannoside.

to the isolated lectin. This clearly indicated that banana extract contains, in addition to *BanLec-I*, other components against which IgG4 antibodies are produced, but that the lectin is a dominating antigen.

Inhibition of Banana IgG4 RAST by BanLec-I

More direct evidence for the dominating role of the lectin amongst the antigens in the banana extract was obtained by an inhibition experiment. The panel of ten serum samples and a normal human serum sample were inhibited by *BanLec-I* and tested for the residual binding of IgG4 antibodies to banana extract. Most sera were largely inhibited by *BanLec-I* (fig. 7). Two sera, Nos. 2 and 5, have IgG4 antibodies to other components in banana extract besides *BanLec-I*, as was confirmed in a test with lectin-depleted banana extract (data not shown).

Inhibition by High-Molecular-Weight Glycoproteins and the Contrainhibitory Effect in the Presence of Mannoside

During the course of this investigation, we observed an unexpected effect of mannoside on the binding of the lectin to IgG4; rather than inhibiting this interaction, mannoside proved to counteract the inhibition of the binding of

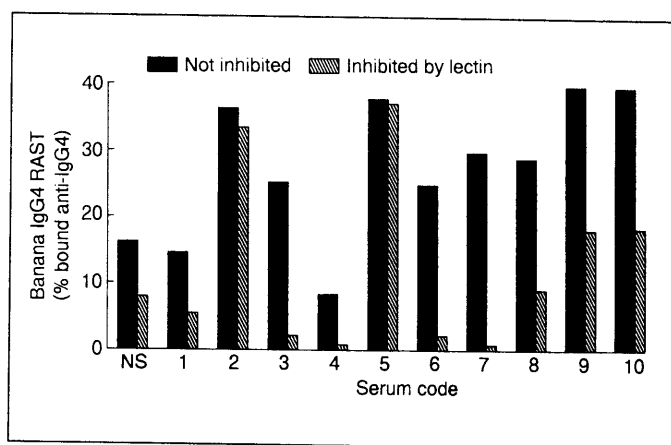


Fig. 7. Inhibition of banana IgG4 RAST by *BanLec-I*. Ten sera and a normal human serum (NS) were inhibited by *BanLec-I*, then incubated with banana Sepharose, and the bound IgG4 was measured with radiolabeled monoclonal anti-IgG4. The bars show the IgG4 antibodies detected with or without inhibition by the lectin.

the lectin to IgG4 by high-molecular-weight glycoproteins. This positive effect of mannoside was further investigated, particularly because this effect was also found with antibodies induced by immunization of rabbits with the lectin. The above-mentioned ten serum samples, a normal human sample, and a hyperimmune rabbit serum were incubated with protein A-Sepharose, and this was further incubated with radiolabeled *BanLec-I* in the presence or absence of sources of high-molecular-weight glycoproteins, such as soybean, crab, or oyster extract. An almost total inhibition of the binding of radiolabeled *BanLec-I* and IgG was obtained (fig. 8). However, when the incubation buffer contained as little as 20 mM of mannoside, the inhibitory effect of the high-molecular-weight glycoproteins was lost, and the original binding of the radiolabeled *BanLec-I* to the IgG was fully reinstated (fig. 8). The presence of galactose, a sugar for which the lectin has no affinity, had no influence on the inhibitory effect of the glycoproteins.

Discussion

The important issue is whether the IgG4-lectin interaction found in the presence of a high concentration of mannoside is an antigen-antibody interaction (hypothesis 1) or, alternatively, a lectin-glycoprotein interaction. It might be argued that perhaps the lectin has only a relatively weak interaction with mannoside and a very strong inter-

action with some sugar configuration present on IgG4 which then might not be susceptible to inhibition by mannoside (hypothesis 2a). Alternatively, the lectin might have two different sugar-binding sites, one for mannoside-type sugars and one for the IgG4-sugar configuration (hypothesis 2b).

We have three arguments in favour of hypothesis 1, i.e., the antibody hypothesis: (1) the contrainhibition effect; (2) the uniqueness of the mannoside-resistant interaction between IgG4 and *BanLec-I*, and (3) the finding that only a small fraction of the IgG4 binds to the lectin.

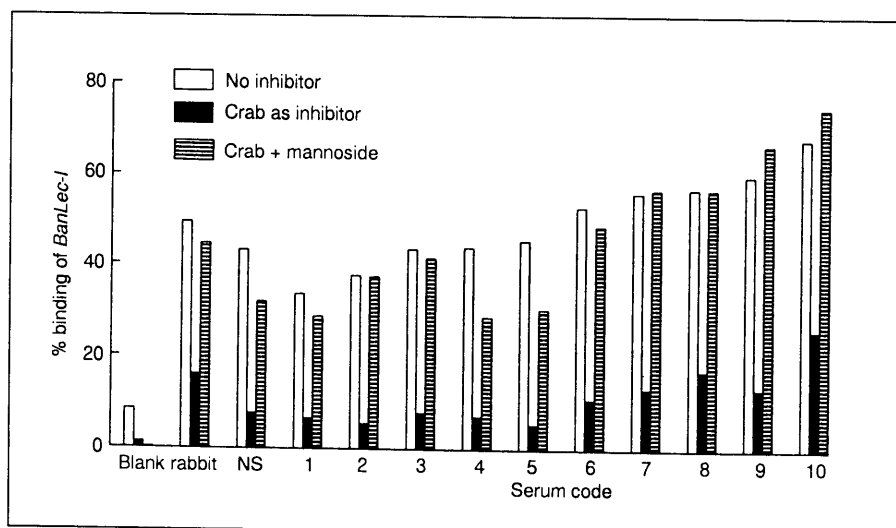
(1) A major argument against hypothesis 2a is the contrainhibition effect of mannoside with both IgG4 and rabbit antibodies. Since mannoside obviously has an effect on the lectin-IgG4 binding, albeit not an inhibitory effect, this clearly indicates that blockade of the mannoside-binding site does occur even in the presence of IgG4 and thus that the mannoside-binding site cannot be involved in IgG4 binding. To illustrate this point, we suggest a hypothetical model of the binding of IgG4, high-molecular-weight glycoprotein, and mannoside to the *BanLec-I* (fig. 9). In this model, the antibody-binding site is located in the proximity of the carbohydrate-binding site. In the presence of a high-molecular-weight glycoprotein, the carbohydrate site is occupied, and in doing so, the high-molecular-weight glycoprotein blocks the antibody-binding site. In the presence of mannoside, the binding of the high-molecular-weight lectin substrate to the carbohydrate-binding site on the lectin is prevented, and the antibody-binding site becomes available to the antibody.

(2) The concept of a mannoside-resistant interaction between glycoproteins and *BanLec-I* (hypotheses 2a/2b) is challenged by the observation that amongst the hundreds of glycoproteins tested, from widely different sources [4], only the binding to IgG4 (and occasionally to other immunoglobulin isotypes) was mannoside resistant. It seems highly unlikely that a small fraction of immunoglobulins has a unique sugar configuration. It is much more likely that the binding to the lectin occurs not via the sugars of IgG4, but via the antigen-binding site. The observation that the lectin binds to the Fab rather than to the Fc part of the IgG also suggests that the antigen-binding site is involved.

(3) The finding that only a small fraction of IgG4 (5% in strongly positive sera, less than 0.01% in negative sera) is lectin reactive and that none of the five IgG4 myeloma proteins tested were positive also supports hypothesis 1: that lectin-positive IgG4 is antibody.

When we incubated nitrocellulose blots of human serum protein separated agar gel electrophoresis with radio-

Fig. 8. Inhibitory effect of crab extract and counterinhibitory effect of mannoside. Ten sera, pooled normal human serum (NS), and a hyperimmune rabbit antiserum to banana were incubated with protein A-Sepharose, followed by incubation with radiolabeled *BanLec-I* in the presence or absence of crab extract. The inhibition of crab extract was carried out with or without mannoside in the test system.



labeled lectin, we found a striking binding to the complement component C3. The binding to other serum proteins was much weaker (data not shown). Since C3 is known to interact with a large variety of macromolecules [6], it is not immediately clear which of the two proteins is the capturing agent: C3 or *BanLec-I*. Most likely, the interaction occurs via the carbohydrate-binding site of the lectin, since incubation of the nitrocellulose blot with mannoside, subsequent to the binding of the labeled lectin, removed the lectin from C3 (but not from the immunoglobulin fraction).

There is good evidence that C3 is involved in the regulation of the immune response, particularly during the maturation phase [6]. The normal antibody response to foods is characterized by the induction of partial tolerance soon after the initial response. One possible explanation for the abnormal immune response to *BanLec-I* might be that the interaction of the lectin and C3 prevents the normal tolerance induction and induces the progression of the immune response to IgG4 antibody production; IgG4 antibody is known to be produced only late during the immune response [1].

A completely different hypothetical explanation for the remarkably high level of antibody to this lectin is that these antibodies are induced by a different mannoside-binding protein, e.g., a mannoside-binding protein present on the pili of gut bacteria [7]. The binding of these antibodies to the banana lectin would then be due to the cross-reactivity between the mannoside-binding proteins. Prelimi-

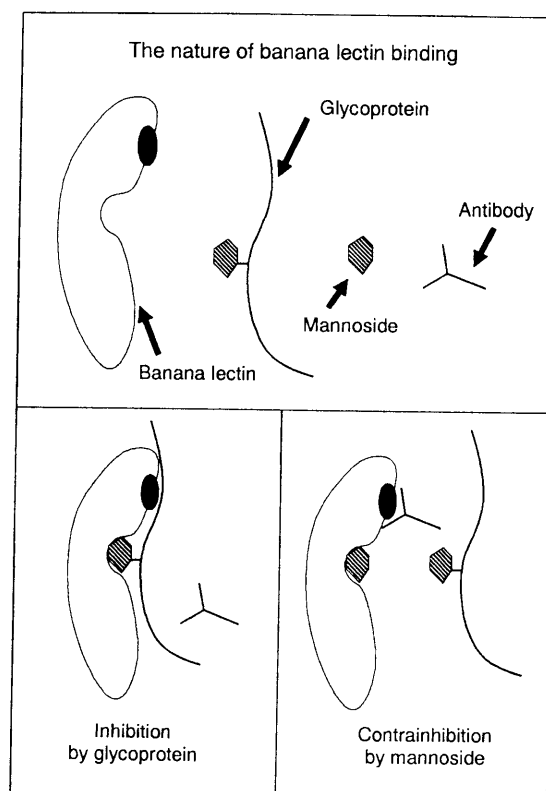


Fig. 9. A schematic model to illustrate the binding of antibody to *BanLec-I*. Top: the molecules participating in the binding; bottom left: inhibition of the binding of IgG4 molecules to the *BanLec-I* by high-molecular lectin substrates; bottom right: the counterinhibition of high-molecular lectin substrate by mannoside, resulting in the availability of the antigen-binding site for the IgG4 molecule.

nary experiments, in which pooled human serum was absorbed with the pili of *Escherichia coli* (kindly provided by Dr. L. van Alphen) and subsequently tested for the *BanLec-I*-binding activity, did not support this explanation.

The most attractive explanation of our findings, however, is that these results support the suggestion by de Aizpura and Russell-Jones [8] that some lectins are particularly prone to induce an immune response upon oral feeding. These authors found that lectins are excellent carrier proteins for inducing an antihapten response by oral immunization. Their explanation is that these lectins bind to intestinal mucosal cells in a way that promotes a strong systemic immune response. It is of interest that

both in their animal model as well as in our results the antibody response is predominantly IgG rather than IgA.

The banana lectin might, therefore, be a potentially useful protein for oral antihapten immunization in humans.

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