Mode of Complement Activation by Acidic Heteroglycans from the Leaves of *Artemisia princeps* PAMP

Haruki YAMADA,* Takayuki NAGAI, Jong-Chol CYONG, and Yasuo OTSUKA

Oriental Medicine Research Center of the Kitasato Institute, 5–9–1, Shirokane, Minato-ku, Tokyo 108, Japan. Received January 31, 1991

The mode of action of the anti-complementary acidic heteroglycans, AAF-Iib-2 and Iib-3 which consisted of rhamnogalacturonan core and arabinogalactan moieties, purified from the leaves of *Artemisia princeps* PAMP (Japanese name = Gaiyo) were investigated. The anti-complementary activities of AAF-Iib-2 and Iib-3 were reduced partially in the absence of Ca^{2+} ions. A marked consumption of C4 was observed to have occurred when serum was incubated with both polysaccharides in the presence of Ca^{2+} ions. AAF-Iib-2 showed more potent C4 consumption than Iib-3. After the incubation of the serum with AAF-Iib-2 in the absence of Ca^{2+} ions, a cleavage of C3 in the serum was detected by immunoelectrophoresis. AAF-Iib-2 showed more significant consumption of the complement than Iib-3 when rabbit erythrocytes were used in the assay system in the absence of Ca^{2+} ions. These results indicate that AAF-Iib-2 activates the complement *via* both the alternative and classical pathways, whereas Iib-3 mainly activates the complement *via* the classical pathway. The absorption of serum with Protein A-Sepharose results in a decrease of the activity of AAF-Iib-2 and Iib-3. However, the decrease of the activity was restored by the replacement of the immunoglobulin G (IgG) fraction after its recovery from the Protein A-Sepharose. These results suggest that IgG dependent mechanisms are both involved in the anti-complementary activity of AAF-Iib-2 and Iib-3.

Keywords *Artemisia princeps*; anti-complementary activity; polysaccharide; action mode; immunoglobulin G

The complement system plays an important role in host defence, inflammation or allergic reactions, and activation occurs *via* both the classical and alternative pathways. The classical pathway is activated by an immune complex containing immunoglobulin M (IgM) and IgG antibodies, the acute phase protein such as C-reactive protein, and ribonucleic acid (RNA) tumor viruses. The alternative pathway is directly activated by polysaccharides, certain immunoglobulins, viruses, fungi, bacteria, certain animal cells and parasites. Some anti-complementary polysaccharides, for example, lipopolysaccharide (LPS),¹⁾ β -(1→3)glucan,²⁾ 6-branched β -(1→3)glucan²⁾ and inulin,³⁾ have already been isolated from bacteria, fungi and plants. Potent anti-complementary activity has also been observed in the extracts of some Chinese herbs.⁴⁾ One of these, the extract of the leaves of *Artemisia princeps* PAMP also contained potent anti-complementary activity.⁵⁾ *Artemisiae Argyi Folium*, the leaves of *A. princeps* PAMP (Japanese name = Gaiyo) is a well known crude drug clinically used in the treatment of colic pain, vomiting and diarrhea, and irregular bleeding from the uterus. In previous papers, we have reported the purification and structural characterization of the anti-complementary acidic polysaccharides, AAF-Iib-2 and Iib-3, from the leaves of *A. princeps* PAMP.^{5,6)} AAF-Iib-2 and Iib-3 are suggested to be structurally related acidic heteroglycans which contained rhamnogalacturonan and arabinogalactan moieties in different proportions.⁶⁾

The present paper describes the mode of action of these anti-complementary polysaccharides.

Materials and Methods

Purification of Anti-complementary Polysaccharides, AAF-Iib-2 and Iib-3, from *A. princeps* PAMP AAF-Iib-2 and Iib-3 were purified from the leaves of *A. princeps* PAMP as reported previously.⁵⁾ The crude polysaccharide was prepared by hot-water extraction and dialysis, and was purified by ion exchange chromatography on diethylaminoethyl (DEAE)-Sepharose, affinity chromatography on *Ricinus communis*-agglutinin conjugated Sepharose and gel filtrations on Sephadex G-100 and Sepharose CL-4B.

Anti-complementary Activity The anti-complementary activity was measured as described previously.⁷⁾ Gelatin-veronal-buffered saline (pH

7.4) containing 500 μM Mg^{2+} and 150 μM Ca^{2+} (GVB^{++}) was prepared as previously described, and normal human serum (NHS) was obtained from a healthy adult. Various dilutions of AAF-Iib-2 or Iib-3 in water (50 μl) were incubated with 50 μl of NHS and 50 μl of GVB^{++} . The mixtures were incubated at 37°C for 30 min and the residual total hemolytic complement (TCH_{50}) was determined by a method using IgM-hemolysin-sensitized sheep erythrocytes (EA) at 1×10^8 cells/ml. NHS was incubated with water and GVB^{++} to provide a control. The anti-complementary activity of the polysaccharide was expressed as the percentage inhibition of the TCH_{50} of the control.

Determination of the Complement Hemolysis through the Alternative Complement Pathway (ACH_{50}) ACH_{50} was determined⁸⁾ in 10 mM EGTA (ethyleneglycol-bis(β -aminoethylether)*N,N,N',N'*-tetraacetic acid) containing 2 mM MgCl_2 in GVB^{--} (Mg^{2+} -EGTA- GVB^{--}). A sample of AAF-Iib-2 or Iib-3 was incubated with Mg^{2+} -EGTA- GVB^{--} and NHS at 37°C for 30 min, and the residual complement of the mixtures was measured by the hemolysis of rabbit erythrocytes (5×10^7 cells/ml) incubated with Mg^{2+} -EGTA- GVB^{--} .

Crossed Immunoelectrophoresis NHS was incubated with a half volume of the solution of the anti-complementary polysaccharide with Mg^{2+} -EGTA- GVB^{--} for 30 min at 37°C. The serum was then subjected to crossed immunoelectrophoresis to locate the C3 cleavage products.⁹⁾ Shortly after the first run (barbital buffer pH 8.6, ionic strength 0.025, with 1% of agarose), the second run was carried out on a gel plate (2.0 mm layer) containing 3% of a rabbit anti-human C3 serum at a potential gradient of 0.8 mA/cm for 15 h. After the electrophoresis, the plate was fixed and stained with Ponceau 3R.

Determination of C4 Titration of C4 was performed¹⁰⁾ using intermediate cells EAC1^{IP} for C4. EAC1^{IP} cells were prepared from EA (1×10^9 cells/ml) incubated with C1 solution (1×10^{12} SFU/ml) in the ratio of 28:1 at 4°C for 1 h.

Analytical Procedures The total carbohydrate content was determined by the phenol-sulfuric acid method,¹¹⁾ using glucose as the respective standard. Protein was assayed by measuring the absorption at 280 nm.

Affinity Absorption of NHS NHS (2 ml) was applied to an asialofetuin-Sepharose 4B column (1.26 $\times$ 2.0 cm) and washed with veronal-buffered saline (pH 7.3) containing 500 μM Mg^{2+} and 150 μM Ca^{2+} (VBS^{++}) at 4°C. The asialofetuin-unbound fraction was used for the measurement of anti-complementary activity. NHS (2 ml) was also applied to a Protein A-Sepharose CL-4B column (1.26 $\times$ 1.7 cm) and washed with VBS^{++} at 4°C. The Protein A-unbound fraction was used for the measurement of anti-complementary activity. IgG was eluted from the Protein A-Sepharose column with 0.05 M sodium citrate (pH 4.5), and dialyzed against VBS^{++} . The TCH_{50} values of both asialofetuin-Sepharose-passed serum and Protein A-Sepharose-passed serum were the same as that of Sepharose passed serum.

Immunoabsorption of Protein A-Unbound Fraction The wells of 96-well polystyrene plate (Sumitomo Bakelite Co., Ltd.) were coated with 100 μl of $\text{F}(\text{ab})_2$ fragment of rabbit anti-human IgG (0.7 mg/ml as antibody

protein) in 0.01 M sodium bicarbonate (pH 9.6) containing 0.02% NaN_3 by incubation at 4°C for 4 h. The wells washed three times with 0.01 M phosphate-buffered saline, pH 7.2 (PBS^-). Then the Protein A-unabsorbed fraction was added to the wells, and the filled plate was incubated at 0°C for 24 h. The resulting IgG-depleted solution was used for the measurement of anti-complementary activity.

Materials Protein A-Sepharose CL-4B, CNBr-activated Sepharose 4B, and Sepharose 4B and CL-4B were obtained from Pharmacia-LKB Biotechnology, and Bio-Gel P-2 (200–400 mesh) from Bio-Rad. F(ab')_2 fragment of rabbit anti-human IgG was purchased from Cappel Lab. Inc. (U.S.A.). Fetuin was purchased from Sigma. Asialofetuin was prepared by mild acid hydrolysis (80°C, 1 h) and dialysis.¹²⁾ Asialofetuin-Sepharose 4B was prepared by the coupling of asialofetuin and CNBr-Sepharose 4B.¹³⁾ An anti-complementary polysaccharide, LR-polysaccharide IIa as a positive control, was purified from the root of *Lithospermum euchromum* ROYLE.¹⁴⁾

Results

Mode of Action of Anti-complementary Polysaccharides, AAF-IIb-2 and IIb-3 AAF-IIb-2 and IIb-3 were shown to have potent dose-dependent anti-complementary activity, and AAF-IIb-2 was more active than IIb-3 (Fig. 1). However, the anti-complementary activities of these polysaccharides were less in the absence of Ca^{2+} ions with surplus Mg^{2+} ions (Fig. 1). When NHS was incubated with

Fig. 1. Change of TCH_{50} by Incubation with AAF-IIb-2 or AAF-IIb-3 in the Presence or Absence of Ca^{2+} Ions

AAF-IIb-2 in GVB^{++} (●), AAF-IIb-2 in Mg^{2+} -EGTA- GVB^{--} (○), AAF-IIb-3 in GVB^{++} (▲), AAF-IIb-3 in Mg^{2+} -EGTA- GVB^{--} (△).

Fig. 2. Consumption of C4 by AAF-IIb-2 or AAF-IIb-3 Treatment
AAF-IIb-2 (●), AAF-IIb-3 (▲), LR-polysaccharide IIa (■).

AAF-IIb-2 or IIb-3 in GVB^{++} at 30°C for 30 min and the residual activity of C4 estimated by hemolytic assay, AAF-IIb-2 was shown to decrease the C4 content of NHS drastically (Fig. 2). About 100% of the hemolytic titer of C4 was consumed by the use of 100 μg/ml of AAF-IIb-2, and this result was similar to the consumption by LR-polysaccharide IIa, an anti-complementary polysaccharide from *L. euchromum* ROYLE. AAF-IIb-3 also decreased the C4 content of NHS significantly: when NHS incubated with 100 μg/ml of AAF-IIb-3 was used for C4 titration, ca. 50% of the hemolytic titer of C4 was consumed. These results show that these polysaccharides can activate the complement via the classical pathway, and AAF-IIb-2 was shown to be twice as active as IIb-3. When these polysaccharides were incubated with NHS in Mg^{2+} -EGTA- GVB^{--} at 37°C for 30 min and the residual complement titer was determined by hemolytic assay using rabbit erythrocytes (ACH_{50}), AAF-IIb-2 and IIb-3 also showed anti-complementary activity on ACH_{50} (ACP activity) (Fig. 3). AAF-IIb-2 was more active than IIb-3.

Fig. 3. ACP Activity of AAF-IIb-2 or AAF-IIb-3
AAF-IIb-2 (○), AAF-IIb-3 (▲).

Fig. 4. C3 Activation by AAF-IIb-2 or AAF-IIb-3 Treatment

NHS was incubated with a half volume of (A) PBS^- , or 1 mg/ml of (B) AAF-IIb-2, (C) AAF-IIb-3 and (D) LR-polysaccharide IIa solution with Mg^{2+} -EGTA- GVB^{--} at 37°C for 30 min. The sera were then subjected to crossed immunoelectrophoresis to locate C3 cleavage products. The anode is to the left.

Therefore, crossed immunoelectrophoresis was carried out after the incubation of NHS with these polysaccharides in Mg^{2+} -EGTA-GVB $^{--}$ to determine whether C3 activation had occurred (Fig. 4). A cleavage of the C3 precipitin line was observed in the serum treated with AAF-Iib-2, but AAF-Iib-3 could not cleave C3 significantly. These results indicate that AAF-Iib-2 also activates the complement *via* the alternative pathway.

Effects of Serum Factors on the Anti-complementary Activity of AAF-Iib-2 and Iib-3 To know how these polysaccharides activate the complement, we examined the effects of serum factors on the anti-complementary activity. AAF-Iib-2 and Iib-3 contained galactose as a predominant component sugar.⁵⁾ Therefore, we first examined the possibility as to whether some galactose specific lectin-like molecule in the serum is involved in the activity. After serum was passed through an asialofetuin-Sepharose 4B column at 4°C, an unabsorbed fraction was used for the measurement of the anti-complementary activity of AAF-Iib-2 and Iib-3. However, the activity of the use of this fraction did not change in comparison with that by the use of Sepharose 4B column-passed serum as a control (data not shown). Asialofetuin is possessing Gal- β -(1→4)-GlcNAc as the non-reducing terminal structure of *N*-linked sugar chains and Gal- β -(1→3)-GalNAc as *O*-linked sugar chain. Therefore, this result denied the possibility of the involvement of galactose binding protein in serum having specificity for these galactosyl structures to the appearance of anti-complementary activity by AAF-Iib-2 and Iib-3. Next, serum was passed through a Protein A-Sepharose CL-4B column at 4°C, and an unabsorbed fraction was used for the measurement of the anti-complementary activity of AAF-Iib-2 and Iib-3. As shown in Figs. 5a and b, both AAF-Iib-2 and Iib-3 decreased their anti-complementary activity dramatically by the use of this serum, comparing with the use of Sepharose CL-4B column-passed serum. When Protein A-Sepharose-absorbed fraction was

added to the Protein A-Sepharose-passed serum again and the resulting serum was used for measurement of anti-complementary activity, the activity was recovered to a similar level with the activity by the use of NHS (Fig. 5c). These results suggested that IgG molecules are involved in the appearance of the activity by the polysaccharides. When these polysaccharides were measured for anti-complementary activity by the use of Protein A-Sepharose-passed serum, the remaining significant activity was still observed. Therefore, to know whether this remaining activity was caused by the IgG molecule unabsorbed to the Protein A-Sepharose, this Protein A-Sepharose-passed serum was absorbed with anti-human IgG antibody. However, no more reduction of the activity was observed (Fig. 5d). When Protein A-Sepharose-passed serum was used for measurement of consumption of C4 by AAF-Iib-2 and Iib-3, the consumption of C4 by both polysaccharides was decreased partially comparing with the use of Sepharose passed serum (data not shown).

Discussion

We have purified two kinds of anti-complementary acidic polysaccharides, AAF-Iib-2 and Iib-3, from the leaves of *A. princeps* PAMP.⁵⁾ AAF-Iib-2 and Iib-3 were composed of rhamnose, xylose, arabinose, galactose, glucose, galacturonic acid and glucuronic acid in the molar ratios of 7.6:7.6:13.0:10.9:3.0:51.2:6.7 and 3.9:2.6:24.7:19.7:2.6:15.0:31.5, respectively.⁶⁾ AAF-Iib-3 has a main chain consisting of (1→4)-linked galacturonic acid and (1→2)-linked rhamnopyranosyl residues which is mostly substituted with arabino-3,6-galactan and arabino-4-galactan at position 4 of rhamnosyl residues.⁶⁾ Most of the arabinosyl residues of arabino-3,6-galactan in AAF-Iib-3 exist in α -L-furanosyl form and are mostly attached to position 3 of (1→6)-linked galactosyl residues as in the non-reducing terminals and highly branched side chains.⁶⁾ The basic structure of AAF-Iib-3 is similar to that of Iib-2, but AAF-Iib-3 has a higher arabinogalactan content than Iib-2.^{5,6)} AAF-Iib-3 activated the complement mainly *via* the classical pathway. The mode of action of AAF-Iib-3 was similar to that of another anti-complementary polysaccharide, AR-arabinogalactan (AG) Iib-1, which was isolated from the root of *Angelica acutiloba* KITAGAWA.¹⁵⁾ The structure of AAF-Iib-3 was also similar to that of AGIib-1. AGIib-1 is a complex arabinogalactan consisting of four carbohydrate units; one neutral and two acidic arabinogalactans and one neutral arabinan, which are linked to each other by acid labile linkage.^{15b,c)} The acidic arabinogalactan units in AGIib-1 consist of a rhamnogalacturonan core possessing at least three kinds of arabinogalactan side chains.^{15b,c)} When the neutral arabino-3,6-galactan unit in AGIib-1 was digested with exo- α -arabinofuranosidase, the activity was markedly enhanced through the alternative pathway.^{15d)} These results suggest that the arabinofuranosyl side-chains of AAF-Iib-3 might inhibit the expression of activity through the alternative pathway. AAF-Iib-2 showed more potent activation of the complement *via* both the classical and alternative pathways than Iib-3. The mode of action of AAF-Iib-2 was similar to that of LR-polysaccharide IIa (acidic heteroglycan) from the root of *L. euchromum* ROYLE,¹⁴⁾ Plantago-mucilage A (partially *O*-acetylated

Fig. 5. Effects of Protein A-Sepharose and Anti-IgG Treatments of the Serum on Anti-complementary Activity of AAF-Iib-2 (A) or AAF-Iib-3 (B)

Sepharose-passed serum (a), Protein A-Sepharose-passed serum (b), Protein A-Sepharose-passed serum plus Protein A-Sepharose-absorbed fraction (c) or anti-IgG-treated Protein A-Sepharose-passed serum (d) was incubated with an equal volume of indicated concentration of AAF-Iib-2 or AAF-Iib-3 solution with GVB $^{++}$ at 37°C for 30 min. The sera were then subjected to total hemolytic complement (TCH₅₀) assay.

glucuronoarabinofuranoxylan) from the seed of *Plantago asiatica*,^{16b,17)} and AR-2IIId (pectin) from the root of *A. acutiloba* KITAGAWA.¹⁸⁾ AR-2IIId contained polygalacturonan regions and "ramified" regions consisting of rhamnogalacturonan cores with β -(1 $\rightarrow$ 6)-linked galactooligosaccharides and a trace of arabino-3,6-galactan moiety.¹⁸⁾ AAF-IIB-2 also has a low arabino-3,6-galactan,⁶⁾ and it may be related to the mode of action of AAF-IIB-2. But further studies of the structure and the anti-complementary activity are required to clarify the more detailed correlation of structure and mode of activity.

It is known that lipid A,¹⁹⁾ RNA virus,²⁰⁾ heparin-protamine complex²¹⁾ and C-reactive protein-C-polysaccharide complex²²⁾ bind to C1q directly, and enhance the activation of the C1 molecule by antibody independent mechanism. Ihara *et al.*²³⁾ find complement activating antimicrobial protein (camp) in several animal sera. Camp has a specific affinity to the carbohydrate moieties of LPS in the presence of Ca^{2+} and Mg^{2+} ions.^{23,24)} Camp activates the complement *via* the classical pathway,²⁵⁾ and is a different molecule from several classes of IgG molecule.²⁶⁾ Therefore, we examined whether a lectin-like molecule or IgG is involved in the anti-complementary activity by AAF-IIB-2 and IIB-3, but an asialofetuin-Sepharose-passed fraction did not reduce the activity. When Protein A-Sepharose-passed serum was used for the measurement of the anti-complementary activity by AAF-IIB-2 and IIB-3, the activity was much reduced, and recovered by the addition of the absorbed fraction into the IgG-depleted serum. These results indicate that the IgG molecule or some natural antibody is involved in the appearance of the anti-complementary activity of AAF-IIB-2 and IIB-3. It is known that sera of various animals contain "natural" antibodies to carbohydrate components such as blood type antibody.²⁷⁾ Sela *et al.*²⁸⁾ find that normal sera from various animals contained anti-carbohydrate IgG antibodies which are purified by fetuin-Sepharose. Because asialofetuin-Sepharose treatment of NHS did not diminish the activity by AAF-IIB-2 and IIB-3, we do not know if the IgG molecule in our experiment is related to this anti-carbohydrate antibody. Schenkein and Ruddy²⁹⁾ also reported that naturally occurring IgG antibody to zymosan contributes to the function of the alternative pathway by enhancing the rate of deposition of C3 from the fluid phase to the zymosan surface and thus increasing the rate of formation of the alternative pathway C3 convertase. Recently, Galili *et al.*³⁰⁾ reported that all humans (except for immunosuppressed patients) are producing a large amount of anti-galactosyl IgG as a natural antibody in their sera, and bacteria within normal intestinal flora may provide constant antigenic stimulation for the synthesis of this antibody. Pectic arabinogalactans and pectins are characteristically associated with the cell wall of both higher and lower plants, and several of these polysaccharides have been isolated from many kinds of plants, such as corn,³¹⁾ soybean,³²⁾ jujube,³³⁾ cabbage³⁴⁾ and rice.³⁵⁾ Therefore, when humans take vegetables and/or fruits as foods, it is possible to synthesize the anti-polysaccharide antibodies. But, we do not know yet whether anti-galactosyl natural antibody in human serum recognizes these plant polysaccharides or another anti-plant polysaccharide antibody is present in the serum.

AAF-IIB-2 and IIB-3 still showed anti-complementary activity even when Protein A-unabsorbed serum was used. This observation suggests that both polysaccharides activate the complement by IgG dependent and independent mechanisms. The present results also showed that these polysaccharides activated the complement through the classical pathway by an IgG dependent mechanism, but we do not know the involvement of this mechanism to the activation of the alternative pathway. Further studies on this subject are now in progress.

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References

- 1) H. Gewurz, H. S. Shin and S. E. Mergenhagen, *J. Exp. Med.*, **128**, 1049 (1968); R. L. Marcus, H. S. Shin and M. M. Mayer, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 1351 (1971); L. Pillemer, M. D. Schoenberg, L. Blum and L. Wurzel, *Science*, **122**, 545 (1955).
- 2) T. Okuda, Y. Yoshioka, T. Ikekawa, G. Chihara and K. Nishioka, *Nature New Biol.*, **238**, 59 (1972); J. Hamuro, U. Hadding and D. Bitter-Suermann, *Immunology*, **34**, 695 (1978).
- 3) O. Götze and H. J. Müller-Eberhard, *J. Exp. Med.*, **134**, 90s (1971).
- 4) M. Shindoh, J.-C. Cyong, H. Yamada, H. Kiyohara and Y. Otsuka, *Proc. Symp. Wakan-yaku*, **16**, 76 (1983).
- 5) H. Yamada, K. Ohtani, H. Kiyohara, J.-C. Cyong, Y. Otsuka, Y. Ueno and S. Omura, *Planta Med.*, **1985**, 121 (1985).
- 6) H. Yamada, Y. Otsuka and S. Omura, *Planta Med.*, **1986**, 311 (1986); H. Yamada, H. Kiyohara and Y. Otsuka, *Carbohydr. Res.*, **170**, 181 (1987).
- 7) a) H. Yamada, H. Kiyohara, J.-C. Cyong, Y. Kojima, Y. Kumazawa and Y. Otsuka, *Planta Med.*, **50**, 163 (1984); b) H. Yamada, H. Kiyohara, J.-C. Cyong and Y. Otsuka, *Mol. Immunol.*, **22**, 295 (1985).
- 8) T. A. E. Platts-Mills and K. Ishizaka, *J. Immunol.*, **113**, 348 (1974).
- 9) J.-C. Cyong, S. S. Witkin, B. Rieger, E. Barbarese, R. A. Good and N. K. Day, *J. Exp. Med.*, **155**, 587 (1982).
- 10) M. E. Wilson and D. C. Morrison, *Eur. J. Biochem.*, **128**, 137 (1982).
- 11) M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
- 12) R. G. Spiro and V. D. Bhoyroo, *J. Biol. Chem.*, **249**, 5704 (1974).
- 13) P. Cuatrecasas, *J. Biol. Chem.*, **245**, 3059 (1970).
- 14) H. Yamada, J.-C. Cyong and Y. Otsuka, *Int. J. Immunopharmacol.*, **8**, 71 (1986).
- 15) a) H. Yamada, H. Kiyohara, J.-C. Cyong and Y. Otsuka, *Carbohydr. Res.*, **159**, 275 (1987); b) H. Kiyohara, H. Yamada and Y. Otsuka, *ibid.*, **167**, 221 (1987); c) H. Kiyohara and H. Yamada, *ibid.*, **193**, 173 (1989); d) H. Kiyohara, J.-C. Cyong and H. Yamada, *ibid.*, **193**, 193 (1989).
- 16) a) M. Tomoda, M. Takahashi and S. Nakatsuka, *Chem. Pharm. Bull.*, **21**, 707 (1973); b) H. Yamada, T. Nagai, J.-C. Cyong, Y. Otsuka, M. Tomoda, N. Shimizu and K. Shimada, *Carbohydr. Res.*, **144**, 101 (1985).
- 17) M. Tomoda, M. Yokoi and K. Ishikawa, *Chem. Pharm. Bull.*, **29**, 2877 (1981); M. Tomoda, N. Shimizu, K. Shimada, R. Gonda and H. Sakabe, *ibid.*, **32**, 2182 (1984).
- 18) H. Kiyohara, J.-C. Cyong and H. Yamada, *Carbohydr. Res.*, **182**, 259 (1988); *idem*, *ibid.*, **193**, 201 (1989).
- 19) N. R. Cooper and D. C. Morrison, *J. Immunol.*, **120**, 1862 (1978).
- 20) N. R. Cooper, F. C. Jensen, R. M. Welsh Jr. and M. B. A. Oldstone, *J. Exp. Med.*, **144**, 970 (1976).
- 21) A. B. Fiedel, R. Rent, R. Myhrman and H. Gewurz, *Immunology*, **30**, 161 (1976).
- 22) M. H. Kaplan and J. E. Volanakis, *J. Immunol.*, **112**, 2135 (1974).
- 23) I. Ihara, Y. Harada, S. Ihara and M. Kawakami, *J. Immunol.*, **128**, 1256 (1982).
- 24) M. Kawakami, I. Ihara, S. Ihara, A. Suzuki and K. Fukui, *J. Immunol.*, **132**, 2578 (1984).
- 25) I. Ihara and M. Kawakami, *Microbiol. Immunol.*, **25**, 751 (1981).
- 26) M. Kawakami, I. Ihara, A. Suzuki and Y. Harada, *J. Immunol.*, **129**, 2198 (1982).
- 27) A.-M. Staub and P. Grabar, *Ann. Inst. Pasteur*, **69**, 368 (1943); J. L.

- Turk, *Br. J. Exp. Pathol.*, **40**, 97 (1959).
- 28) B.-A. Sela, J. L. Wang and G. M. Edelman, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 1127 (1975).
- 29) H. A. Schenkein and S. Ruddy, *J. Immunol.*, **126**, 7 (1981); *idem*, *ibid.*, **126**, 11 (1981).
- 30) U. Galili, R. E. Mandrell, R. M. Hamadeh, S. B. Shohet and J. M. Griffiss, *Infect. Immun.*, **56**, 1730 (1988).
- 31) Y. Kato and D. J. Neins, *Plant Physiol.*, **74**, 562 (1984).
- 32) G. O. Aspinall, K. Hunt and I. M. Morrison, *J. Chem. Soc., C*, 1080 (1967).
- 33) N. Shimizu and M. Tomoda, *Chem. Pharm. Bull.*, **31**, 499 (1983).
- 34) B. H. J. Stevens and R. R. Selvendran, *Phytochemistry*, **23**, 107 (1984).
- 35) N. Shibuya and R. Nakane, *Phytochemistry*, **23**, 1425 (1984).