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Comparison Between the *in vitro* Interaction of Lectins (PHA and Con A) and Antimicrococcus Antibodies on Normal and Malignant Cells*

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Abstract—*Rabbit sera and purified antibodies directed against* Micrococcus lysodeikticus *(a Gram-positive, non-pathogenic and easy-to-eliminate bacterium) do not bind at 37°C to autologous, isologous or heterologous (mouse and human) erythrocytes, as measured by agglutination assays, heterologous (guinea pig) complement cytotoxicity or Coombs test reactivity. However, rabbit and mice antimicrococcus sera agglutinate Ehrlich carcinoma cells and human leucocytes.*

Both lectins, Con A and PHA, agglutinate Ehlrich carcinoma cells, human leucocytes and erythrocytes of different species but only ConA is able to agglutinate Micrococcus cells. Biological effects of cell surface binding are discussed.

INTRODUCTION

MALIGNANT transformation of animal cells yields a variety of changes such as uncontrolled growth, lowered adhesion, altered mobility and migration, increased rate of nutrient uptake and loss of organization of cytoskeletal components [1]. Cell surface alterations are involved in these changes, which can be detected by the agglutinability of transformed cells, unlike normal cells, at low lectin concentration [2]. Although lectin binding receptor sites were demonstrated on both normal and transformed cells [3], Noonan and Burger [2] found that transformed cells bind 3.5–5 times more [^{3}H]-Concanavalin A (ConA) than does the normal interphase cell. Agglutinability of cells by lectins is related to glycoprotein receptor mobility [4] which depends on morphological structures as microvilli [5], anchored by cytoskeletal structures involving filaments, microfilaments and microtubules. The carbohydrate moiety of glycoproteins is known to be part of the lectin receptor site [6]. Inbar *et al.* [7] have studied the relationship of Con A agglutinability to tumourigenicity using chemically and virally transformed fibroblasts and concluded that highly Con A-agglutinable cells were tumourigenic whereas agglutination by soy bean and wheat germ agglutinin (WGA) failed to correlate with tumourigenicity. Similarly, tumourigenicity and Con A agglutinability were shown to coincide in transformed 3T3 lines [8] or in Chinese hamster embryo lines [9].

When glycopeptides obtained from the surface of normal and transformed cells were compared, Ogata *et al.* [10] provided evidence for addition of whole chains of sialyl-galactosyl-*N*-acetyl-glucosaminyl branches in glycopeptides derived from transformed cells. In addition, Pouysségur *et al.* [11], when studying

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the biochemical reversion of an *N*-acetyl-glucosamine-deficient fibroblast mutant, clearly demonstrated the role of cell surface carbohydrates in cell behaviour.

Normal and immune sera fractionated on a column of immobilized glycoproteins such as fetuin yield antibodies specific for the carbohydrate portion. Because of the capability to agglutinate heterologous erythrocytes and their mitogenicity on lymphocytes [12], these antibodies behave as 'immune lectins'. Shier [13] demonstrated that a 'chemical vaccine' of di-*N*-acetyl-D-glucosamine coupled to poly (-L-asparate) in complete Freund adjuvant enabled mice to reject five times as many transplanted myeloma tumour cells as those rejected by identically treated control mice.

Antibodies raised in rabbits against di-*N*-acetyl-chitobiose coupled to bovine serum albumin failed to agglutinate erythrocytes from various species but did agglutinate transformed (MOPC 173) cells and lymphocytes of mice [14]. Collins and Wust [15] reported that a mixed vaccine of group A *Streptococcus pyogenes* and *Bordetella pertussis* prevents the growth of tumours produced by a line of SV-40-transformed cells in golden Syrian hamsters. They also demonstrated that antibodies to tumour polysaccharide reacted with tumour and *S. pyogenes*' peptidoglycan (polysaccharide), which exhibits antibodies cross-reacting with micrococcus' peptidoglycan [16]. Rabbit antibodies against *Micrococcus lysodeikticus* (ATCC 4698) are of high titer (up to 40 mg/ml serum) [17] and are directed [18] either against the bacterium specific carbohydrate, called Perkins' antigen (a copolymer of glucose and *N*-acetyl-D-mannosaminuronic acid) or against the bacterial peptidoglycan built up by repeating units of *N*-acetyl-D-glucosamine, *N*-acetyl-D-muramic acid and a cross-linking peptide [19–21]. Previously, we demonstrated that hypervaccination of mice with *Micrococcus lysodeikticus* offered a considerable (antibody-mediated) [22] immunoprotection against grafts of leukemic L1210 [23], plasmacytoma (MOPC 173) and Ehrlich carcinoma cells [24]. Since precipitation–inhibition studies have shown that glucose and *N*-acetyl-D-glucosamine, which are specific ligands for lectins such as Con A and WGA [25], are the immunodominant saccharides [18], a study was carried out to determine whether antimicrococcus antibodies interact with erythrocytes of different species *in vitro* and display a similar toxicity or discriminate more selectively between (normal) erythrocytes and cancer cells.

MATERIALS AND METHODS

Materials

Concanavalin A was a 10 % protein powder obtained from Sigma (lot No. 94C 5015). Phytohaemagglutinin was purchased from the Wellcome Company (lot No. K7644). Sigma trypsin (lot No. F8253) with 11,400 B.A.E.E. units/mg were used. U-shaped wells containing agglutination plates were obtained from Linbro Chemicals, New Haven (Code IS-MRC-96). Sterile blood of sheep was obtained from the Pasteur Institute, Brussels. Rabbits were obtained from a local breeder and blood from the ear vein was collected and stored in heparin containing Alsever solution.

Methods

Trypsinolysis of erythrocytes. After repeated washings and centrifugations at 3013 ***g*** for 20 min, 4°C, the erythrocyte pellet was resuspended in the enzyme buffer solution 10 times the original volume. Under frequent shakings, trypsinolysis was performed for 1 hr at 37°C in PBS buffer, (0.01M potassium phosphate—0.15 M NaCl; pH = 7.3) by adding the enzyme at a final 0.1 % concentratation.

Lectin agglutination assay. In Linbro plates, 0.05 ml PBS was pipetted and two-fold consecutive dilutions were made by adding 0.05 ml from an original 1.2 mg/ml Con A or 1 mg/ml PHA solution. After incubation of 30 min at 37°C, 0.05 ml of the repeatedly washed target cell suspension was added at a concentration of 2 mg/ml micrococcus or 10^8 erythrocytes/ml and agglutinations were scored after 1 hr and expressed in $\log_2$ units.

Agglutination of mammalian cells by the anti-micrococcus antiserum. In Linbro plates, consecutive two-fold dilutions were made by adding 0.05 ml of the antiserum to 0.05 ml of PBS. After pre-incubation at 37°C, 0.05 ml of the washed material (10^8 cells/ml) in PBS was added and agglutinations scored after 2 hr. Erythrocytes of rabbit (RRBC) and sheep (SRBC) as well as trypsin treated rabbit (TRRBC) and sheep (TSRBC) were tested. A BALB/c mouse, bearing Ehrlich ascites tumour for 12 days, was killed and tumour cells were aspirated from the peritoneal cavity, washed thrice with ice-chilled PBS and fixed with 0.3 % formaldehyde in PBS for 24 hr at 25°C. After repeated washings, a suspension of 7.21×10^6 Ehrlich cells/ml was made in PBS. This procedure was shown to preserve carefully histocompatibility and other antigenic membrane markers on tumour cells [26].

Characterization of the antiserum. Rabbit anti-

micrococcus antibodies were screened for their antigenic specificity. After removal of non-specifically bound material by 3M NaCl, antibodies were specifically eluted from an immunoabsorbent of micrococcus cell wall coupled to Sepharose 4B, as earlier described [22], either by 0.5 M glucose or 0.5 M *N*-acetyl-D-glucosamine or subsequently by 0.1 M acetic acid. The protein content was determined quantitatively by assuming a spectrophotometric absorbance at 280 nm of A $1\%/1$ cm $= 14$.

Complement cytotoxicity. After repeated washings, rabbit erythrocytes were resuspended in veronal buffered saline supplemented with 0.05% of gelatin (VBSG) at a final 2×10^8 cells/ml concentration. In haemolysis test-tubes, 0.1 ml of the target cell suspensions was added to VBSG. 0.1 ml of the purified anti-micrococcus antibody solutions of known activity was added and shaken for 30 min at 37°C, and 0.05 ml of the undiluted guinea pig complement (Wellcome Company) was added. According to the Terasaki test, commercial vials of guinea pig complement were titrated before use [27]. After shaking for 60 min at 37°C, the unlyzed residual cells were centrifuged at 297 ***g*** for 10 min, 4°C, washed twice with VBSG, centrifuged, and finally lyzed by adding 2 ml of bidistilled H_2O. Free haemoglobin concentration, measured at 415 nm was used as a direct and quantitative estimation of the number of residual unlyzed erythrocytes, after complement mediated haemolysis.

Isolation of specific antimicrococcus antibodies. Two millilitre aliquots of rabbit antimicrococcus sera were applied onto an immuno adsorbent of cell wall-coupled-Sepharose 4B. After washing the column with PBS and removal of non-specifically bound material by 3M NaCl, the antibody fraction was eluted by 0.1M acetic acid and directly neutralized by addition of 1M K_2HPO_4, repeatedly dialyzed against PBS buffer at 4°C, and finally concentrated to the original volume on Amicon B15 concentrators and the protein concentration was determined by measuring the optical density at 280 nm (Zeiss, PMQ II), assuming an absorbance of $A^{1\%}_{1\,cm} = 14$ for immunoglobulins.

The Coombs test for detection of 'incomplete' antibodies. Anti-rabbit IgG serum of donkey (Miles–Yeda: 1.6 mg/ml, code No. 61-090) was kindly supplied by Dr. James Prahl.

The serum was heated at 56°C for 30 min and absorbed with pooled erythrocytes of 6 pre-immune rabbits, according to the procedure of Boyse *et al.* [28]. A quarter of a millilitre of pooled erythrocytes of 6 pre-immune rabbits (2.10^8/ml) were pre-incubated for 30 min, incubated for 1 hr with 0.25 ml of antimicrococcus or pre-immune control sera at 37°C, added after centrifugation and washings to series of consecutive two-fold dilutions of the anti-rabbit IgG serum made in Linbro plates.

Control experiments were run in parallel using sheep erythrocytes coated by rabbit (IgG) antibodies, (sheep hemolysin), at sub-agglutinating level. After standing 2 hr at 37°C, agglutinations were scored and yielded a haemagglutination titer $= 10.5$ ($\log_2$ units) for the controls.

Immunization procedures. Preparation of chitin. micrococcus cell wall and the coupling of oligosaccharides of chitin on this cell wall (hereafter called cell wall-chitin) were performed as earlier described [22].

Rabbits were vaccinated i.v. once a week during 4 weeks with 5 mg of antigen (either micrococcus, chitin or cell wall-chitin). After a rest period of 1 month, animals were boosted i.v. three times a week with 2.5 mg of the antigens during 6 weeks. Rabbits were bled every 2 weeks and their sera prepared.

RESULTS

Con A, unlike PHA, agglutinates micrococcus cells. The agglutination can be completely inhibited by 0.01M glucose and 0.1M *N*-acetyl-D-glucosamine (Fig. 1). Both lectins

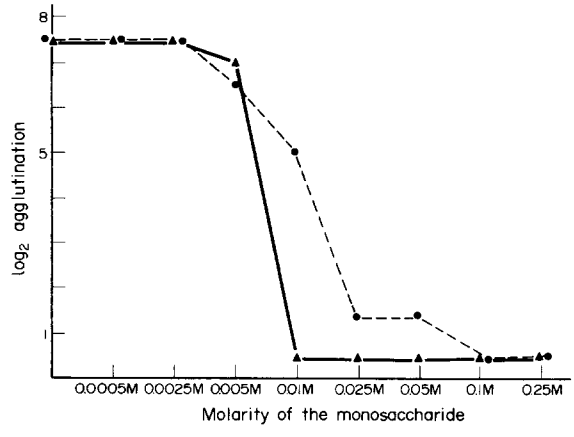

Fig. 1. *Inhibition of the binding of Con A on* Micrococcus lysodeikticus *cells by glucose* (▲—▲) *and N-acetyl-D-glucosamine* (●----●).

Washed micrococcus cells were resuspended in phosphate buffered saline (PBS). In U-shaped Linbro plates, 0.05 ml *of the Con A* (1 mg/ml) *was added to* 0.05 ml *of the monosaccharide and consecutive two-fold dilutions were made in the monosaccharide solution.*0.05 ml *of micrococcus suspension* (1 mg/ml) *was added and agglutination was scored after standing* 4 hr *at* 37°C.

agglutinate rabbit erythrocytes in a temperature dependent fashion. Trypsinization of erythrocytes resulted in an increase of the temperature sensitive agglutination by both lectins and could be inhibited by glucose and *N*-acetyl-D-glucosamine. It is remarkable that agglutinability by PHA is inversely correlated with increasing temperature. Agglutination-inhibition was less pronounced for PHA since the specific ligand (D-galactose) was not used (Table 1).

When tested with a selected panel of rabbit antimicrococcus antisera of homogeneous and non-homogeneous antibody producers, we never observed any agglutination of the complement inactivated antisera with human or mice erythrocytes (not shown) at 37°C. When untreated as well as trypsin treated (0.1%—60 min at 37°C) and washed erythrocytes of rabbit and sheep were tested, agglutination by complement inactivated rabbit antimicrococcus sera was never observed at 37°C. Trypsinization of erythrocytes resulted in an increased agglutinability by both lectins Con A and PHA. Antimicrococcus sera did not agglutinate untreated erythrocytes but agglutination patterns were obtained for isologous trypsinized rabbit (37°C) erythrocytes, as indicated in Table 2.

Because binding of auto-antibodies on autologous cells is an auto-immune process and may lead to auto-immune disease with a detrimental outcome for the host, an intensive study was carried out to investigate carefully whether vaccinations with *Micrococcus lysodeikticus* induced the formation of antisera or more specifically antimicrococcus antibodies, capable of binding to autologous or isologous erythrocytes, at physiological temperature.

We have approached this question by agglutination studies and complement cytotoxicity (a more sensitive technique for detecting interactions at subagglutinating level).

(1) When 10 rabbit antimicrococcus sera of known antibody titer were assayed, agglutination of autologous erythrocytes by complement inactivated antisera was never observed at 37 or 4°C.

(2) When complement inactivated antimicrococcus sera of 41 different rabbits were tested for complement cytotoxicity or for haemagglutination of rabbit erythrocytes, we repeatedly failed to demonstrate any positive interaction with isologous erythrocytes at subagglutinating or agglutination level. However, it is worthy of note that complete haemolysis of rabbit erythrocytes was obtained by mixing rabbit anti-cell wall-chitin and partial hemolysis by mixing rabbit anti-chitin sera with isologous erythrocytes in the presence of guinea pig complement (some of them are shown in Table 3).

(3) We have screened antimicrococcus sera of 10 rabbits during the whole immunization period for haemolytic activity on isologous erythrocytes to make clear whether lytic activity was transiently present. By using a standard residual cell lysis technique, we have determined quantitatively the percentage of residual cells, after guinea pig mediated attack of antimicrococcus sera on isologous erythrocytes. We conclude that haemolysis could never be demonstrated.

(4) We have examined whether specific antimicrococcus antibodies, obtained by elution from an immuno-adsorbant of micrococcus cell wall conjugated to Sepharose 4B, agglutinate isologous rabbit erythrocytes or lyse them in the presence of exogenous complement. In parallel, experiments were run for testing the corresponding antimicrococcus sera. Experiments were performed in triplicate and mean values $\pm$ S.D. are presented. As evidenced in Table 4, haemolysis was never observed. By contrast, anti-chitin antibodies, obtained by elution from the same immuno-adsorbant, induced haemolysis in presence of guinea pig complement.

(5) Some antibodies such as anti-Rhesus immunoglobulins are known to be incomplete [29, 30]. If such antimicrococcus would exist, they still could cause haemolysis with effector mechanisms other than complement (i.e. antibody dependent cellular cytotoxicity). Searching for these antibodies, we have incubated pooled erythrocytes of 6 pre-immune rabbits with 15 rabbit antimicrococcus sera and 5 pre-immune sera for 1 hr and a Coombs agglutination test was performed. No antimicrococcus serum was found to agglutinate rabbit erythrocytes whereas control haemolysin-coated-erythrocytes showed strong agglutination patterns with the Coombs serum ($\log_2 = 10.5$ units).

Since lectins such as Con A agglutinate virtually all tumour cells five times better than normal cells [2], we tested Ehrlich ascites carcinoma cells (EAC) for agglutinability by Con A, PHA and the antimicrococcus panel. As demonstrated in Table 5, rabbit antimicrococcus sera, both homogeneous and heterogeneous, agglutinate fixed EAT at 4 and 37°C. This is unlike the pre-immune sera but similar to the effect observed with lectins. By later experiments, we have developed an immunization schedule that yields the pro-

Table 1. Comparison of agglutinability of normal and trypsin treated rabbit erythrocytes by Con A and PHA

Lectin	Target: erythrocytes	Temperature	Hemagglutination titer (log_2 units)	Lectin	Target: erythrocytes	Temperature	Hemagglutination titer (log_2 units)
Con A (1.2 mg/ml)	Untreated	4°C	9.5 (PBS)	PHA (1 mg/ml)	Untreated	4°C	9.0 (PBS)
idem.	Trypsin treated	4°C	12.0 (PBS)	*idem.*	Trypsin treated	4°C	12.5 (PBS)
			1.0 (0.5M glu)				10.0 (0.5M glu)
			2.0 (0.5M NAG)				10.0 (0.5M NAG)
idem.	Untreated	22°C	12.0 (PBS)	*idem.*	Untreated	22°C	8.0 (PBS)
idem.	Trypsin treated	22°C	16.0 (PBS)	*idem.*	Trypsin treated	22°C	11.0 (PBS)
			4.0 (0.5M glu)				9.5 (0.5M glu)
			8.0 (0.5M NAG)				9.5 (0.5M NAG)
idem.	Untreated	37°C	12.0 (PBS)	*idem.*	Untreated	37°C	7.0 (PBS)
idem.	Trypsin treated	37°C	16.0 (PBS	*idem.*	Trypsin treated	37°C	9.0 (PBS)
			4.0 (0.5M glu)				8.5 (0.5M glu)
			8.0 (0.5M NAG)				8.0 (0.5M NAG)

In U-shaped Linbro agglutination plates, consecutive two-fold dilutions were made by adding 0.05 ml of the lectin PHA (1 mg/ml—Wellcome) or Concanavalin (1.2 mg/ml—Sigma) to 0.05 ml of phosphate buffered saline (PBS). After a preincubation of 30 min, 0.05 ml of the erythrocyte suspension (10^8 cells/ml) or trypsin treated (0.1%—60 min at 37°C) and washed erythrocyte suspension was added and allowed to react at the indicated temperature. Agglutinations in PBS or inhibition of agglutination by glucose (0.5M glu) or *N*-acetyl-D-glucosamine (0.5M NAG) were scored after standing 2 hr.

Table 2. Agglutinability of different rabbit antimicrococcus antisera on untreated or trypsin treated erythrocytes

Serum and character	Micrococcus agglutination titer (log_2 units)	RRBC agglutination titer (log_2 units)	TRRBC agglutination titer (log_2 units)	SRBC agglutination titer (log_2 units)	TSRBC agglutination titer (log_2 units)
202 Pre-immune	0.0	0.0	0.0	0.0	0.0
113 Homogeneous	6.0	0.0	1.5	0.0	0.0
332 Homogeneous	6.5	0.0	0.0	0.0	0.0
132 Heterogeneous	7.0	0.0	1.0	0.0	0.0
322 Homogeneous	13.0	0.0	1.5	0.0	0.0
104 Heterogeneous	7.0	0.0	0.5	0.0	0.0
Con A (Sigma—1.2 mg/ml)	6.5	12.0	16.0	—	—
PHA (Wellcome—1 mg/ml)	0.0	7.0	9.0	—	—

In U-shaped Linbro agglutination plates, consecutive two-fold dilutions were made by adding 0.05 ml of the complement inactivated antiserum of heterogeneous and homogeneous responders to 0.05 ml of PBS. After a preincubation of 30 min, 0.05 ml of the washed material (10^8 cells/ml) in PBS was added. Agglutination was scored after standing 2 hr at 37°C.

Erythrocytes of rabbit (RRBC) and sheep (SRBC) as well as trypsin treated (0.1%—60 min at 37°C) and washed erythrocytes of rabbit (TRRBC) and sheep (TSRBC) were tested.

Table 3. Agglutinability and complement cytotoxicity on rabbit erythrocytes by rabbit antimicrococcus sera

Serum and character	Micrococcus agglutination titer (log_2 units, 37°C)	Haemagglutination titer (log_2 units, 37°C)	Cells lysed by complement (%)
113 Preimmune	1.0	0	0
103 Preimmune	0.0	0	0
208 Antimicrococcus	11.0	0	0
302 Antimicrococcus	8.0	0	0
308 Antimicrococcus	12.0	0	0
210 Antimicrococcus	9.0	0	0
410 Antimicrococcus	14.0	0	0
310 Antimicrococcus	11.0	0	0
303 Antimicrococcus	9.0	0	0
313 Antimicrococcus	5.0	0	0
207 anti-cell-wall-chitin	11.0	3	100
202 anti-cell-wall-chitin	14.5	4	100

In U-shaped Linbro plates, consecutive two-fold dilutions were made by adding 0.05 ml of the complement inactivated rabbit sera to 0.05 ml of PBS. After a preincubation of 30 min, 0.05 ml of the erythrocyte suspension (10^8 cells/ml) or micrococcus suspension (2 mg/ml) were added and allowed to react.
Complement cytotoxicity was assayed by addition of 0.05 ml of undiluted guinea pig serum and the percentage of cells lysed by complement was determined quantitatively by a standard residual cell lysis technique.

Table 4. Agglutinability and complement cytoxicity on rabbit erythrocytes by antimicrococcus antibodies and the corresponding rabbit serum

Serum and character	Micrococcus aggl. titer* (log_2 units, 37°C)	Hemagglutination* (log_2 units, 37°C)	O.D. 415 nm of lysate* of residual cells	O. D. 415 nm of lysate† of residual cells
113 Antimicrococcus	11.0	0.0	1.32 ± 0.05	1.24 ± 0.010
110 Antimicrococcus	8.5	0.0	1.32 ± 0.02	1.16 ± 0.03
332 Antimicrococcus	11.0	0.0	1.34 ± 0.00	1.25 ± 0.07
105 Antimicrococcus	12.0	0.0	1.23 ± 0.01	1.17 ± 0.07
108 Antimicrococcus	17.0	0.0	1.41 ± 0.05	1.25 ± 0.07
360 Antimicrococcus	17.0	0.0	1.28 ± 0.01	1.25 ± 0.01
367 Antimicrococcus	13.0	0.0	1.32 ± 0.05	1.14 ± 0.07
211 Antimicrococcus	11.0	0.0	1.38 ± 0.02	1.25 ± 0.08
364 Antimicrococcus	12.0	0.0	1.31 ± 0.06	1.23 ± 0.01
355 Antimicrococcus	14.0	0.0	1.33 ± 0.06	1.19 ± 0.04
318 Antimicrococcus	15.0	0.0	1.32 ± 0.04	1.25 ± 0.01
361 Antimicrococcus	15.0	0.0	1.30 ± 0.03	1.25 ± 0.05
311 Anti-chitin	7.0	4.0	0.09 ± 0.02	–
Pool of globulins (pre-immune)	1.0	0.0	1.33 ± 0.06	–

In U-shaped Linbro agglutination plates, consecutive two-fold dilutions were made by adding 0.05 ml of the antiserum† or the antibody* fraction to 0.05 ml of PBS. After a pre-incubation of 30 min, 0.05 ml of erythrocytes suspension (10^8 cells/ml) or micrococcus suspension (2 mg/ml) were added and allowed to react.
After complement cytotoxicity, residual unlyzed cells were centrifuged, washed twice with VBSG, lyzed and the absorbance at 415 nm was determined.
Experiments were performed in triplicate and mean values (O.D. 415 nm) $\pm$ S.D. are expressed.

Table 5. Agglutinability of Ehrlich ascites tumour cells (EAC) by lectins and rabbit antimicrococcus sera

Serum and character	Micrococcus agglutination titer (log_2 units)	EAT agglutination titer (log_2 units, 4°C)	EAT agglutination titer (log_2 units, 37°C)
202 Pre-immune	0.0	0.0	0.0
113 Homogeneous	6.0	6.0	5.0
332 Homogeneous	6.5	3.0	3.0
132 Heterogeneous	7.0	4.0	3.0
322 Homogeneous	13.0	4.0	3.0
104 Heterogeneous	7.0	3.0	3.0
Con A (Sigma—1.2 mg/ml)		7.0	7.0
PHA (Wellcome—1.0 mg/ml)		6.5	6.0

Fifty microlitres of complement inactivated rabbit antimicrococcus antisera were added to 0.05 ml of PBS and consecutive two-fold dilutions were made in U-shaped Linbro plates and pre-incubated for 30 min.
A BALB/c mouse, bearing EAC for 12 days, was killed and tumour cells were aspirated from the peritoneal cavity, washed three times with ice-cold PBS and fixed with 0.3% formaldehyde in PBS for 24 hr at 25 °C.
After repeated washings, a suspension of 7.21×10^6 EAC cells/ml was made in PBS.
Fifty microlitres of the cell suspension was added to the Linbro plates and allowed to react. In parallel, lectin preparations (Con A 1.2 mg/ml and PHA 1 mg/ml) were tested.

duction of antimicrococcus antibodies of high titer and restricted electrophoretic heterogeneity in BALB/c and CDF_1 mice. The agglutinability of live EAC by syngeneic mouse antimicrococcus antibodies was demonstrated.

The next experiment was designed to compare lectin with antimicrococcus interactions on human blood cells.

In a first approach, agglutinability of human erythrocytes by PHA and Con A was studied. When untreated and trypsinized (0.1%—60 min at 37°C) erythrocytes of different ABO and Rhesus groups were tested, we found comparable agglutination patterns for both lectins (Table 6). Trypsinization always resulted in an increased agglutinability by both lectins.

In Table 7, we present evidence that human leucocytes can be agglutinated at 37°C by different rabbit antimicrococcus sera. Agglutination of untreated or trypsinized human erythrocytes (i.e. AB, Rh^+) by the antimicrococcus panel did not occur at 37°C. Similarly, mouse erythrocytes were not agglutinated by rabbit antimicrococcus sera at physiologic temperature.

DISCUSSION

In mammals, the immune response to glycoprotein is predominantly directed at protein antigenic epitopes and not at the carbohydrate moiety [31]. Because of the similarity of the carbohydrate moiety of serum glycoproteins and glycoproteins functioning as cell surface receptors, it is probable that animals have acquired selective tolerance and recognize those polysaccharides [6, 32] as 'self antigens'. However, when animals are infected or vaccinated with Gram-positive streptococci [33], pneumococci [34] or non-pathogenic micrococci [17] that carry an analogous carbohydrate as a part of the bacterial cell wall, the elicited anticarbohydrate immune response is abundant and often of restricted electrophoretic and structural geneity (homogeneous antibodies), a characteristic in common with the well-studied myeloma proteins [35]. Since rabbit antibodies against *Micrococcus lysodeikticus* (ATCC 4698), a Gram-positive, non-pathogenic and easy-to-eliminate bacterium (substrate of lysozyme) display antigenic anticarbohydrate specificities (mainly anti-glucose and *N*-acetyl-D-glucosamine) which overlap with ligand specificities of lectins, we have examined whether antimicrococcus display similar antibody-lectin effects on normal and transformed cells. Rabbit antimicrococcus sera or immuno-adsorbent purified antibodies were never able to agglutinate autologous, isologous or heterologous (human) erythrocytes at 37°C. In contrast with experiments using anti-chitin sera, rabbit antimicrococcus sera did not exhibit antibody-mediated haemolysis by guinea pig complement.

Subsequently, experiments were devised to detect possible weak agglutinins or so-called 'incomplete' antibodies by the Coombs test. Again, no binding of antimicrococcus immunoglobulins on erythrocyte cell surfaces could be demonstrated. Consequently, antimicrococcus antibodies are not lectins *sensu stricto*. By contrast, we repeatedly observed that

rabbit antimicrococcus sera as well as syngeneic mouse antimicrococcus agglutinate EAC at physiologic temperature. Agglutinability by rabbit antimicrococcus sera of human leucocytes but not of erythrocytes was shown.

Further experiments indicate that antimicrococcus immunoglobulins may physiologically interfere in the proliferative phase of lectin-stimulated lynphocytes, while not providing a sufficient mitotic triggering signal themselves (Verloes *et al.*, in press).

Evidently, antimicrococcus antibodies constitute a particular class of anticarbohydrate antibodies. They are clearly distinct from specific anti-blood group antibodies (mostly IgM) or from the mitogenic anti-carbohydrate antibodies and are reactive with cell surface carbohydrates and cross-reactive with epitopes on bacteria of the intestinal flora [12].

In search of a reliable marker for malignancy, Bramwell and Harris [36] have recently compared the glycoproteins of plasma membranes from a variety of normal and tumour cells. They noticed that a 100,000 dalton glycoprotein binds substantially larger quantities of Con A and less WGA than the same protein of normal cells, suggesting a specific abnormality in the glycosylation of this protein. This abnormality was present in carcinoma, sarcoma, lymphoma and melanoma cells but not in normal and hybrid non-tumourigenic cells.

The antimicrococcus immunity protects mice against grafts of L1210, plasmacytoma MOPC 173 and EAC tumours [23, 24] and displays an anticarbohydrate specificity (mainly anti-glucose) [18]. Further examinations are needed to investigate whether specific antimicrococcus antibodies may selectively recognize differences in carbohydrates between malignant and normal, parental cells. In search for an immunotherapeutic treatment of cancer, we suggest the possible use of non-pathogenic bacteria as vaccines to elicit substantial amounts of anticarbohydrate antibodies, such as antimicrococcus, which seem to discriminate between normal and tumourigenic cell lines.

Until now, no clear-cut indication for autoimmune pathology of antimicrococcus antibodies has been reported and vaccination for years in rabbits, mice and hare did not reveal any toxicity or apparent morbidity.

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