10 Hanahan, D., and Meselson, M., Gene *10* (1980) 63.
11 Grunstein, M., and Hogness, D.S., Proc. natl Acad. Sci. USA *72* (1975) 3961.
12 Cami, B., and Kourilsky, P., Nucleic Acids Res. *5* (1978) 2381.
13 Denhardt, D.T., Biochem. biophys. Res. Commun. *23* (1966) 641.
14 Rigby, P.J.W., Dieckmann, M., Rhodes, C., and Berg, P., J. molec. Biol. *113* (1977) 237.
15 Southern, E., J. molec. Biol. *98* (1975) 503.
16 Chenciner, N., Meneguzzi, G., Corallini, A., Grossi, M.P., Grassi, P., Barbanti-Brodano. G., and Milanesi, G., Proc. natl Acad. Sci. USA *77* (1980) 975.
17 Galibert, F., Mandart, E., Fitoussi, F., Tiollais, P., and Charnay, P., Nature *281* (1979) 646.
18 Wetmur, J.G., Biopolymers *14* (1975) 2517.
19 Gerhardt, D.S., Kawasaki, E.S., Bancroft, F.C., and Szabo, P., Proc. natl Acad. Sci. USA *78* (1981) 3755.

0014-4754/84/050483-03$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1984

Serum lectins from the scorpion *Vaejovis spinigerus* Wood bind sialic acids[1]

G.R. Vasta[2] and E. Cohen

Medical University of South Carolina, Department of Biochemistry, Charleston (South Carolina 29425, USA), and Roswell Park Memorial Institute, Department of Laboratory Medicine, Buffalo (New York 14263, USA), 27 June 1983

Summary. We have partially characterized the specificity of serum lectins from the scorpion *Vaejovis spinigerus* Wood. Agglutination, crossed-absorption and hemagglutination-inhibition patterns were similar but not identical to serum lectins from other members from the family Vaejovidae, and different from the Buthidae species studied so far. *V. spinigerus* serum lectins bind sialic acids and sialoconjugates, but also bind 2-keto-3-deoxyoctonate, uronic acids and N-acylaminosugars, all substances present in bacterial cell walls suggesting that they might be involved in defense functions.

Interest in the distribution and specificity of humoral lectins in the Subphylum Chelicerata (which comprises the classes Merostomata, Pycnogonida and Arachnida), a very conservative taxon in its evolutive aspects, motivated us to pursue a systematic study of the occurrence and serological properties of serum lectins from North American scorpions, whip scorpions and spiders (Arachnida). At present all arachnid species examined by us exhibited multiple serum lectins some of which are specific for sialic acids[3-7]. In this report we describe the serological characterization of serum lectins from the scorpion *Vaejovis spinigerus* Wood.

Material and methods. Scorpion sera: scorpions *Vaejovis spinigerus:* (18 males and 22 females, *V. confuscius:* 3 males and 2 females) were collected near Mesa, Arizona, and bled from pedipalps. Pooled scorpion hemolymph was allowed to clot at room temperature (25 ± 2 °C), the serum was cleared by centrifugation at 5000 × g for 15 min and stored at −25 °C.

Erythrocytes (RBC): Human blood samples were collected in ACD (citrate-dextrose). All other blood samples from diverse vertebrate species were obtained by venous or cardiac puncture and collected in Alsevers. RBC were washed twice with saline 0.85% and twice with tris-buffered saline: 100 mM tris-HCl, 50 mM NaCl, 10 mM $CaCl_2$, pH 7.6 (TBS), and suspended at concentrations of 5×10^6 RBC/ml in TBS.

Reagents: Neuraminidase from *Vibrio cholerae* (VCN) 500 U/ml and fetuin were purchased from Gibco, Grand Island, New York. N-acetylneuraminic acid β-methyl glycoside (NANA β MeGly) was kindly supplied by Dr W. Korytnyk, Roswell Park Memorial Institute, Buffalo, New York. All other reagents were purchased from Sigma Chemical Co., St. Louis, Missouri, at the highest purity available.

Enzyme treatment of RBC: RBC were treated with pronase P (P)(protease type VI from *Streptomyces griseus,* 3–4 units/mg) and VCN by the procedure of Uhlenbruck et al.[8]. P-treated RH_o(D) human RBC showed satisfactory agglutination titers with an incomplete anti-D serum (Ortho, Raritan, New Jersey). Human RBC were not agglutinated by *Limulus polyphemus* serum after VCN treatment.

Agglutination test: 5 µl of 2-fold serial dilutions of scorpion serum in TBS were placed in Terasaki 96 well trays (Robbins Scientific, Mountain View, California) and equal volumes of RBC suspension were added. The trays were vortex-mixed for 10 sec at speed 1 and incubated at room temperature for 45 min. Agglutination was read under the microscope and graded from 0 (negative) to 4+. Titers were recorded as the inverse ratio of the highest dilution showing a ½+ degree of agglutination. Controls for all titrations were the substitution of sera by TBS.

Desialylation of glycoproteins: glycoproteins were dissolved in 0.025 N H_2SO_4, 0.85% NaCl at 10 mg/ml. Hydrolysis was carried out for 1 h at 80 °C. Released sugars were analyzed by paper chromatography in ethyl acetate:pyridine:acetic acid: H_2O (5:5:1:3) for neutral sugars[9] using 1 µg of D-galactose, D-glucose, D-mannose (D-Man) and L-fucose (L-Fuc) as standards developing with the $AgNO_3$ and alcoholic KOH reagents[10], and in n-butanol:n-propanol: 0.1 N HCl (1:2:1) for sialic acids[11] using 1 µg of N-acetylneuraminic acid (NANA) and N-glycolylneuraminic acid (NGNA) as standards, developing with the resorcinol reagent. Sialic acids were the only sugars detected in the chromatograms. No neutral hexoses were released as measured by the anthrone reagent[12]. Released and total sialic acids were determined by the thiobarbituric acid[13] and resorcinol[14] methods respectively.

Percentages of released sialic acids were as follows: bovine submaxillary mucin (BSM): 72.1%; fetuin: 78.1%; PSM: 99.1%; thyroglobulin: 83.3%; bovine orosomucoid: 96.1%, and human orosomucoid: 98.3%. Hydrolyzed glycoproteins were exhaustively dialyzed against 0.85% NaCl and TBS, aliquoted and stored at −25 °C.

Hemagglutination-inhibition tests: Scorpion serum was diluted to 4–8 agglutination units/ml (1 agglutination unit is the amount of lectin present in the highest serum dilution which agglutinates with a degree of 2 in the scale from 0 to 4). All substances to be tested were dissolved in TBS (at concentrations up to 200 mM for mono- and oligosaccharides and 1% (w/v) for polysaccharides and glycoproteins) and brought to pH 7.6 with concentrated NaOH. Equal volumes of diluted sera and inhibitor solution were mixed and incubated for 45 min at room temperature. The mixtures were titrated as described before, with untreated or enzyme-treated RBC. Minimal concentrations required for the inhibition of two agglutination units were recorded. Controls were the substitution of

Experientia 40 (1984), Birkhäuser Verlag, CH-4010 Basel/Switzerland

Table 1. Hemagglutination profiles of *Vaejovis* sera*

	V. spinigerus			*V. confuscius*		
	U	P	VCN	U	P	VCN
Human A_1	512	8192	0	256	16	0
B	512	8192	0	128	16	0
C	512	2048	0	128	16	0
Dog	512	2048	0	64	8	0
Rabbit	128	0	0	16	32	0
Sheep	4	1024	0	0	16	0
Bovine	0	4096	0	0	0	0
Cat	8	128	0	0	0	0
Goat	0	0	0	0	0	0
Baboon	0	0	0	0	0	0
Stumptail	0	0	0	0	0	0
Rhesus	0	0	0	0	0	0
Horse	2048	2048	2048	512	128	256
Rat	2048	2048	2048	512	128	1024
Mouse	1024	1024	1024	512	32	256
Duck	64	512	0	32	32	0
Chicken	32	1024	0	32	32	0
Pigeon	64	256	0	–	–	–

* Figures are agglutination titers. Titers were recorded as the inverse ratio of the highest dilution showing a ½ + degree of agglutination. U, untreated RBC; P, pronase-treated RBC, VCN, neuraminidase-treated RBC; –, not tested.

the inhibitor solution by TBS, and as described before, substitution of the sera by TBS.

Results and discussion. In table 1 *V. spinigerus* and *V. confuscius* agglutination profiles for untreated and enzyme-treated RBC are compared. Human ABO blood groups were not discriminated and VCN-treated human RBC were no longer agglutinated by *Vaejovis* lectins suggesting that cell surface sialic acids are involved in the carbohydrate moieties recognized by both *Vaejovis* species. However, except for rabbit RBC, *V. spinigerus* titers were increased or remain unchanged after P-treatment of RBC while for *V. confuscius* titers were decreased by P-treatment suggesting that P-sensitive sialopeptides are the structures recognized by the latter. Although all vertebrates species tested are known to exhibit cell surface sialic acids some RBC were not agglutinated by *Vaejovis* lectins; moreover, both *Vaejovis* species discriminated between human and non-human primate RBC. *V. spinigerus* lack of agglutination with untreated bovine RBC was not surprising since these RBC have an hydrophilic glycoprotein coating which prevents exposure of many surface antigens[15]. Lack of agglutination of untreated and enzyme-treated bovine RBC has already been observed with other scorpion lectins[3]. With bird RBC both *Vaejovis* species exhibited agglutination patterns typical of sialic acid specific lectins. Crossed absorption experiments (table 2) showed that multiple specificities were present in *V. spinigerus* serum. Duck and human RBC crossreacted but did not absorb any activity for horse or rat RBC. P-treated horse RBC appeared to be agglutinated by a lectin different from the one which agglutinated the VCN-treated cells. Rat RBC absorbed all lectins suggesting that carbohydrate moieties recognized by all lectins are present on their cell surface. Hemagglutination-inhibition experiments showed that 2-keto-3-deoxyacids, mainly sialic acids, were the best inhibitors of *V. spinigerus* lectins. The trisaccharide sialyllactose was the only compound which inhibited the agglutination of all cells tested. 2-Keto-3-deoxyoctonate was a potent inhibitor only when testing agglutination of human RBC. Other acidic sugars such as uronic acids also inhibited at moderate concentrations. Other N-acetylaminosugars such as GalNAc and ManNAc only inhibited the agglutination of VCN-treated horse RBC. Inhibition by untreated and desialylated glycoproteins indicated that sialic acids were responsible for the inhibitory capabilities of the glycoproteins tested: only sialoglycoproteins inhibited *V. spinigerus* agglutinins. Except for agglutination of VCN-treated horse RBC, which was inhibited (although at high concentrations) by desialylated BSM and thyroglobulin, for all other cells only the untreated glycoproteins inhibited effectively.

Qualitative and quantitative differences in the inhibitory capabilities of most glycoproteins tested depending on the cell and/or enzyme treatment tested corfirmed the heterogeneity of *V. spinigerus* lectins observed in the crossed-absorption experiments. It is interesting that lectins which agglutinated horse and rat RBC could discriminate between orosomucoids of human and bovine origin. Moreover, BSM was a better inhibitor than fetuin to a degree which could not be accounted for by the differences in their sialic acid content. In addition asialo-BSM which retains 27.9% of the sialic acid (SA) content of

Table 2. Crossed absorption test on *V. spinigerus* serum*

V. spinigerus serum dil. 1:10 absorbed with		*V. spinigerus* serum dil. 1:10 tested with: Human P	Duck P	Horse P	Horse VCN	Rat P	Rat VCN
None		512	64	256	256	256	128
Human	P	0	8	128	256	256	128
Duck	P	0	0	256	256	128	128
Horse	P	0	64	0	8	256	256
	VCN	0	8	256	0	128	64
Rat	P	0	0	0	0	0	0
	VCN	0	0	4	0	0	0

* Figures are agglutination titers. Titers were recorded as the inverse ratio of the highest dilution showing a ½ + degree agglutination. P, pronase-treated RBC; VCN, neuraminidase-treated RBC.

Table 3. Hemagglutination-inhibition profiles of *V. spinigerus* agglutinins

Inhibitors[a,b]	Erythrocytes/enzyme treatment: Human P	Horse P	Horse VCN	Rat P	Rat VCN
[mM]					
NANA	6.2	25.0	–	25.0	–
NGNA	6.2	25.0	–	–	–
NANAβMeGly	12.5	25.0	–	50.0	–
Sialyllactose	12.5	50.0	50.0	12.5	50
KDO	6.2	–	–	–	–
GluA	12.5	50.0	–	–	–
GalA	50.0	50.0	–	–	–
GalNAc	–	–	50.0	–	–
ManNAc	–	–	50.0	–	–
[% w/v]					
BSM	0.001	0.007	0.003	0.007	0.007
Asialo BSM	–	–	0.25	–	–
Fetuin	0.003	0.06	0.50	0.25	–
Orosomucoid (bovine)	0.003	0.015	0.06	0.06	0.25
Orosomucoid (human)	0.007	0.12	–	0.25	–
PSM	0.06	–	–	–	–
Thyroglobulin	0.06	–	–	–	–

Figures are minimal concentrations (mM or % w/v) that inhibit 2 agglutination units.

[a] Abbreviations: P, pronase-treated; VCN, Neuraminidase-treated; NANA, N-acetylneuraminic acid; NGNA: N-glycolylneuraminic acid; NANA β MeGly, N-acetylneuraminic acid β-methylglycoside; KDO, 2-keto-3-deoxyoctonate; GluA, glucuronic acid; GalA, galacturonic acid; GalNAc, N-acetyl-D-galactosamine; ManNAc, N-acetyl-D-mannosamine; BSM, bovine submaxillary mucin; PSM, porcine stomach mucin; —, no inhibition at concentrations up to 200 mM (mono-and oligosaccharides) or 1% w/v (glycoproteins).

[b] L-fucose, D-mannose, D-mannosamineHCl, N-acetylglutamic acid, N-acetyl-D-glucosamine, colominic acid, D-galactose, D-glucose, α-lactose, stachiose, raffinose, cellobiose, gentiobiose, D-allose, methoxyneuraminic acid, arabinogalactan, asialo-fetuin, asialo-orosomucoid (bovine or human) asialo-PSM, asialo-thyroglobulin, and ovomucoid did not inhibit at concentrations up to 200 mM (mono- and oligosaccharides) or 1% w/v (glycoproteins and polysaccharides).

untreated BSM, was still a better inhibitor than fetuin for fractions agglutinating VCN-treated horse RBC suggesting that the terminal oligosaccharide SA-α-2-6-GalNAc, abundant in BSM, is preferred to SA-α-2-3(6)-Gal present in fetuin and thyroglobulin. However, since free GalNaC inhibited those lectin fractions it is possible that subterminal GalNAc, exposed by cleavage of sialic acid, was responsible for the inhibitory capabilities of asialo-BSM. The fraction agglutinating human RBC appears to recognize both structures to the same degree. Purification and physicochemical characterization of these lectin fractions now in progress in our laboratory, might confirm some of our tentative conclusions and elucidate possible relationships between specificity and molecular structure.

Although they present minor differences in their agglutination, crossed absorption and agglutination-inhibition profiles, the members of the family *Vaejovidae* studied so far (*Paruroctonus mesaensis, Vaejovis confuscius* and *V. spinigerus*), exhibit serum lectins which bind sialic acids. These profiles are different from the ones observed in the two members, of the Buthidae[3,4] examined in this respect, which, in addition to sialoconjugate-binding lectins also exhibit lectins specific for galactosyl residues. Since sialic acids are a late development in evolution[16], clues about the biological function(s) of chelicerate sialic acid-binding serum lectins might be found in the binding to KDO, uronic acids and N-acylaminosugars, all substances found in bacterial cell walls. These observations suggest that chelicerate serum lectins might be involved in defense functions against bacterial infection.

1 We wish to thank Dr H.L. Stahnke, Arizona State University, Tempe, Arizona, for taxonomic assistance. This work received fellowship support from the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.
2 Recipient of the Fogarty International Fellowship (NIH) 05TWO31155-01.
3 Vasta, G.R., and Cohen, E., Devl. comp. Immun. *6* (1982) 219.
4 Vasta, G.R., Ilodi, G.H.U., Cohen, E., and Brahmi, Z., Devl. comp. Immun. *6* (1982) 625.
5 Vasta, G.R., and Cohen, E., Experientia *39* (1983) 721.
6 Vasta, G.R., and Cohen, E., Comp. biochem. Physiol., in press.
7 Vasta, G.R., and Cohen, E., J. invert. Path., in press.
8 Uhlenbruck, G., Rothe, A., and Pardoe, G.I., Z. Immunforsch. exp. Ther. *136* (1968) 79.
9 Li, E., Tabas, I. and Kornfeld, S., J. biol. Chem. *253* (1978) 7762.
10 Trevelyan, W.E., Proctor, D.P., and Harrison, J.S., Nature *166* (1950) 444.
11 Svennerholm, E., and Svennerholm, L., Nature *181* (1958) 1154.
12 Roe, J.H., J. biol. Chem. *212* (1955) 335.
13 Aminoff, D., Virology *7* (1959) 355.
14 Svennerholm, L., Biochim. biophys. Acta *24* (1957) 604.
15 Pardoe, G.I., Friberg, S., and Greenland, T.B., Rev. Inst. Pasteur Lyon *6* (1973) 373.
16 Warren, L., Comp. biochem. Physiol. *10* (1963) 15.

0014-4754/84/050485-03$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1984

Prolongation of the survival of skin grafts in mice by PUVA treatment

S. Gruner, C. Riese, S. Schnitzler, H. Meffert and E. Karasek

Abt. Klinische Immunologie und Hautklinik, Bereich Medizin (Charité) der Humboldt-Universität, Postfach 170, DDR-1040 Berlin, and Abt. für Versuchstierzucht des Forschungszentrums für Molekularbiologie und Medizin der Akademie der Wissenschaften der DDR (German Democratic Republic), 28 January 1983

Summary. The combined application of psoralen and UVA radiation to skin grafts induced a prolongation of the survival time of the grafts in mice. This was observed using the H-Y barrier, an allogeneic barrier without H-2 disparities, and a strong H-2 incompatible barrier. The effect is probably due to a reduction of antigen-presenting cells, or to other, unknown mechanisms.

During recent years, the so called PUVA therapy (psoralen + UVA irradiation) became popular in the treatment of psoriasis. It was shown that ultraviolet radiation (UVR) has a profound influence on the immune system, especially on antigen-presenting cells like Langerhans cells in the skin or dendritic and interdigitating cells in lymphoid organs. Thereby UVR often induces suppressor T cells and a suppression of immune reactions like contact hypersensitivity or tumor immunity. These questions have been reviewed recently[1-4]. Here we describe experiments to prolong the survival of skin grafts in mice by treatment of the grafts with 8-methoxy-psoralen (8-MOP) and longwave ultraviolet (UVA) irradiation.

Materials and methods. Mice of the strains AB/Bln, BALB/c, DBA/2 Bln, and C57Bl/6 Bln from the breeding colony of the Academy of Sciences of the GDR were used. 8-MOP was purchased from GEROT Pharmaceutica Vienna as a 0.15% solution (Oxsoralen®).

The tails of the donors were smeared twice with a mixture of equal parts of Oxsoralen® and glycerine. After 1 h the donors were anesthetised with 0.2–0.3 ml of a 1.4% solution of hexobarbital sodium salt and irradiated under fluorescent UV lamps (VEB NARVA Brand-Erbisdorf, 20 W, 220 V) that emit mainly UVA light (7% UVB) with an intensity of 2.25×10^{-3} J/cm^2 × sec at a distance of 10 cm. Therefore, the UVR doses were 4.05, 8.10, 16.2, and 32.4 J/cm^2, respectively.

Then small epidermal grafts were excised with a blade and transplanted to similarly prepared skin sites on the recipient's tail. Each recipient received 2 grafts which were covered with a glass tube fixed on the distal end of the tail. The grafts were checked daily for viability using a stereomicroscope. A full rejection was recorded for 1 individual when it had rejected both grafts. For statistical evaluation the nonparametric Wilcoxon Mann-Whitney test was used.

Results. Transplantation against a H-Y barrier: Male donor mice of the inbred strain AB were treated with 8-MOP, the grafts were irradiated in situ with UVR and transplanted to female recipients of the same strain. The results are shown in table 1. They show a remarkable dose-dependent prolongative effect of the PUVA treatment.

Transplantation against an allogeneic barrier without H-2 disparities: Similar experiments were performed using female DBA/2 mice as donors and female BALB/c recipients (both H-2^d). The results are shown in table 2. The mean survival time (MST) showed that also in this system there was a clear-cut dose-dependent prolongative effect of the PUVA treatment on graft survival.

Transplantation against a strong H-2 incompatible barrier: Here, we studied the effect of PUVA treatment in a strong allogeneic barrier, representing a full house mismatch in all transplantation loci. As donors we used C57Bl/6 mice (H-2^b),