Coelenterate nematocysts bind immunoglobulins[1]

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Summary. Nematocyst capsules of *Actinia equina* were shown to bind immunoglobulins. Binding is not affected by EDTA-pretreatment or incubation with N-acetyl-galactosamine and N-acetyl-glucosamine.

In the immunofluorescent localization of cellular compounds in invertebrate tissues, one often is confronted with the problem of nonspecific binding of antibodies. Several reports have dealt with the development of methods to prevent nonspecific fluorescence, e.g. mild pretreatment of frozen sections with various fixatives.

Adsorption of Ig may either be due to nonspecific protein-protein-interactions or to particular receptor molecules which usually bind to carbohydrate containing residues located on the Fc-part of the Ig-molecule. Such receptor molecules have been isolated from a variety of plants, from the albumin gland of *Helix pomatia*[3], from the hemolymph of giant clams[4], and from sponge tissue[5]. Due to their hemagglutinating activity, these substances have been collectively termed phytohemagglutinins or lectins. Next to their ability to agglutinate particular blood cells and antibody-coated erythrocytes, some of these substances act as potent mitogens on lymphocytes. The binding specificity of such lectins, which are now widely used in the study of polysaccharides and surface receptors of cell membranes, has been studied extensively.

Although the general function of lectins still remains unknown, it is suggested that they play a decisive role in processes where specific recognition is the fundamental event in a series of determination and differentiation. The homology of sponge agglutinins and cell aggregation factors has been proposed[5].

Protein A from *Staphylococcus aureus* has been shown to exhibit specific receptor sites for the binding of immunoglobulins[6].

During a course of immunohistochemical localization of contractile proteins in coelenterates[7], we observed a strong fluorescence of *Actinia equina* nematocysts in frozen sections after treatment with FITC-labelled immunoglobulins (table, a). The fluorescence of the capsules was completely abolished by pretreatment with a non-labelled non-immune serum (table, c). It was further shown that fluorescence was not affected by pretreatment of the samples

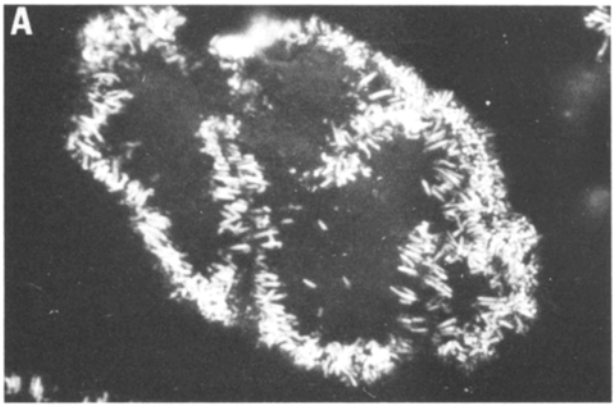

Figure A. Cryostat cross-section of a tentacle of *Actinia equina* (4 µm). Nematocyst staining by FITC-labelled IgG. × 340.

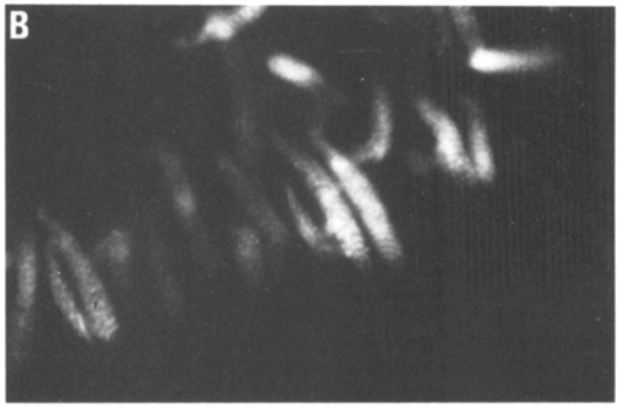

Figure B. Detail from figure a at higher magnification, showing individual nematocysts. Note intense fluorescence of coiled threads and less intense fluorescence of the capsule-wall. × 2125.

The IgG-binding properties of *Actinia equina* nematocysts

1st incubation	2nd incubation	3rd incubation	Fluorescence
a) FITC-rabbit-IgG	–	–	+ +
b) Rabbit-IgG	Goat-anti-rabbit-FITC	–	+ +
c) Goat-IgG	Rabbit-IgG	Goat-anti-rabbit-FITC	– –
d) Rabbit-IgG	Goat-IgG	Goat-anti-rabbit-FITC	+ +
e) $F(ab)_2$-fragment of rabbit-IgG	FITC-rabbit-IgG	–	+ +
f) $F(ab)_2$-fragment of rabbit-IgG	Goat-IgG	Goat-anti-rabbit-FITC	– –
g) Bovine serum albumin (10 mg/ml)	FITC-rabbit-IgG	–	+ +
h) FITC-rabbit-IgG × GalNAc 10 mg/ml	–	–	+ +
i) FITC-rabbit-IgG + GlcNAc 10 mg/ml	–	–	+ +
k) FITC-rabbit-IgG + 10 mM EDTA	10 mM EDTA	–	+ +
l) FITC-rabbit-IgG + 10 mM $CaCl_2$	10 mM $CaCl_2$	–	+ +

IgG-concentrations were 2 mg/ml in phosphate buffered saline; in the presence of Ca^{2+}, TRIS-buffers were used instead. Incubations were carried out for 45 min at room temperature, followed by several washings in phosphate buffered saline. FITC-labelled goat-a-rabbit was obtained from NORDIC. Rabbit IgG was prepared by a conventional method[11] from whole serum.

either with bovine serum albumin (table, g) or with the $F(ab)_2$ fragments of IgG (table, e and f), suggesting that nematocysts have specific receptors on their surface for the Fc-part of immunoglobulins. The presence of N-acetyl-glucosamine or N-acetyl-galactosamine (table, h and i), both of which are known to be the binding substrates of a variety of lectins[8], did not markedly change these binding properties.

Furthermore, the Ig-binding properties of nematocysts are species-nonspecific, since a heterologous IgG will inhibit the binding of FITC-labelled 2nd antibody (table, c and d). The same experiment rules out the possibility of nonspecific FITC-binding to nematocyst membranes.

Nematocysts have been described to be negatively charged, since they are basophilic[9]. This would provide a possible explanation for the nonspecific binding of basic proteins. However, immunoglobulins exhibit isoelectric points near neutrality.

Possibly due to their acidic nature, membrane components of nematocysts have a high affinity for divalent cations which may be substituted by cobalt[10]. However, it has been shown by these authors that at least 60% of the total amount of calcium in nematocysts are tightly bound to the membrane and cannot be removed by prolonged EDTA-treatment. This corroborates the finding that EDTA does not alter the Ig-binding properties of nematocyst membranes (table, k), nor are they affected by high concentrations of calcium which may compensate an overall negative charge of the nematocysts. It is noteworthy that sponge lectins exhibit similar cation-binding properties[5].

The results of the table indicate that the Fc-part of immunoglobulin is specifically bound to the nematocyst surface. At present, we cannot prove whether the carbohydrate moiety of Ig is responsible for the binding to nematocysts, which may contain lectins or lectin-like substances. Further studies including isolation and characterization of the Ig-receptor, e.g. by affinity chromatography will help to elucidate the structure and function of particular nematocyst components.

As can be seen in the figure, the binding of fluorescent immunoglobulins provides a simple and useful technique for the demonstration of nematocysts in frozen sections or smear preparations.

1 This work was supported by the Deutsche Forschungsgemeinschaft (Ste 105/19-21).
2 I wish to thank Drs Ute Groeschel-Stewart, Darmstadt, and Helmut Schuhmacher, Bochum, for helpful discussion.
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Toxic effects of Zn^{++} and Cu^{++} on mouse blastocysts in vitro[1]

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Summary. Mouse blastocysts were cultured in the presence of zinc and/or cupric chloride at various concentrations. Cupric ions were superior to Zn^{++} at inhibiting hatching of blastocysts from their zona pellucida and formation of trophoblastic outgrowths. Protein in the medium protected embryos from the toxic effects of zinc and copper.

Excess zinc ingestion by animals leads to an apparent copper deficiency while excess copper intake results in symptoms of zinc deficiency[2-7]. These toxic effects of excess zinc or copper can be ameliorated by supplementation with the 'deficient' mineral[4-7]. It has been proposed that this Zn-Cu interaction may occur at one or more of several loci including gastrointestinal or cellular absorption and incorporation of the minerals into enzymes, structural proteins and storage compounds[6,7]. I.p. injection of copper sulfate leads to an increase in zinc binding compounds in the liver of rats[8]. Thus excess copper could lead to increased zinc storage and an apparent deficiency state. Moreover, copper and zinc interfere with the intestinal absorption of each other[9,10]. However, it is not yet known whether or not competition for cellular transport sites or for binding sites on proteins, such as enzymes, contributes significantly to the antagonism between these minerals. The latter possibility could best be tested using a cell culture system so that intestinal absorption and storage of the elements would not be complicating factors.

We have used the growth and apparent survival of preimplantation mouse blastocysts in vitro to test for possible antagonism between these 2 minerals. The toxicity of cupric ions to these embryos in culture has been described[11-13] and it is well known that making intrauterine devices out of copper increases their efficacy[14,15]. Moreover, we determined that zinc can inhibit growth and development of blastocysts in vitro. Zinc wire IUD's probably interfere with pregnancy in rats by preventing implantation[15]. Nevertheless, rat uterine fluid normally contains approximately 120 μM Zn[16]. If Zn^{++} and Cu^{++} were equally toxic then the latter concentration of zinc should be toxic to embryos[11-13]. We also determined if the mere presence of Cu^{++} (100 μM) would prevent the implantation-like event of trophoblastic outgrowth in vitro as suggested by the studies of Naeslund[12].

Materials and methods. Random bred Swiss mice (8–12 weeks old) were induced to ovulate and mate with gonadotropins[17]. At approximately 87 h post coitus, blastocysts were flushed from excised uteri with Dulbecco's Modified