S-100-like immunoreactivity in a planarian
An immunochemical and immunocytochemical study

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Summary. The presence of an S-100-like immunoreactivity was investigated in the planarian Dugesia gonocephala. By microcomplement fixation assay, measurable amounts of S-100-like immunoreactive material (0.11 μg/mg soluble protein) were detected in planarian high-speed supernatants. The index of immunological dissimilarity between ox S-100 and planarian S-100-like immunoreactive material was higher than that previously calculated between ox S-100 and all the vertebrates tested. By the immunohistochemical PAP method, S-100-like immunoreactivity was only detectable in the cilia of the epidermal cells. Although the biological meaning of S-100-like immunoreactivity in planarian remains to be clarified, the present data introduce new perspectives into the investigation of S-100.

Key words: S-100-like immunoreactivity – Planarian – Microcomplement fixation – PAP method

The S-100 protein (Moore 1965) is known to be located in definite neuroectodermal cell types of the nervous system (for review see Bock 1978; Zomzely-Neurath and Walker 1980), and also of non-nervous organs (Møller et al. 1978; Cocchia and Miani 1980; Nakajima et al. 1980; Cocchia and Michetti 1981; Cocchia et al. 1981). It has been reported to be present in the cytoplasm as well as in the nucleus, both in a soluble and in a bound form. During the ontogenesis the pattern of accumulation of S-100 parallels the functional maturation of the nervous system, though its biological role remains to be clarified. In the phylogensis the protein has been shown to maintain close immunological relationships in all species of vertebrates tested (for review see Bock 1978; Zomzely-Neurath and Walker 1980). On the other hand, detailed information is lacking on its possible presence in lower species, although preliminary data on the immunological detection of S-100 in invertebrates (namely lobster, crayfish, octopus, cockroach and drosophila) have been reported (Moore 1969).

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The present study deals with an S-100-like immunoreactivity detected in the planarian *Dugesia gonocephala*.

**Materials and methods**

Planarians *Dugesia gonocephala* s.l. (*Platyhelminthes, Turbellaria, Tricladida*), in the agamous scissiparous form, were a kind gift of Dr. V. Margotta (Istituto di Anatomia Comparata, Università di Roma).

*Preparation of antisera.* A specific antiserum to S-100 protein from ox brain was obtained from rabbit and characterized according to Zuckerman et al. (1970). The serological specificity of the antiserum was shown by double diffusion in agar and by quantitative microcomplement fixation analysis employing soluble protein extracts of a variety of tissues as antigens. Sheep antiserum to rabbit IgG was produced by intramuscular injection of a total of 600 mg IgG, as described by Sternberger et al. (1970). Chromatographically pure immunoglobulins from rabbit serum and from sheep antiserum against rabbit IgG were prepared as described by Harboe and Ingild (1973) and their dilution was referred to the starting volume of the respective serum. For control experiments, preimmune rabbit serum or anti-S-100 absorbed as described below with purified ox S-100 were employed. Anti-S-100 antiserum was incubated for 1 h at 37°C with S-100 antigen (100 µg/ml) and the antigen-antibody reaction was continued overnight at 4°C. The procedure was repeated once more with an additional treatment of 10 µg/ml of antigen. The completeness of the reaction between antigen and antibody was evaluated by quantitative precipitin determination and by immunodiffusion (Harboe and Ingild 1973). Peroxidase-antiperoxidase (PAP) complex (1.1 mg protein/ml), prepared according to the procedure of Sternberger et al. (1970), was a gift of Prof. N. Miani (Istituto di Anatomia Umana, Università Cattolica, Roma).

*Purification of S-100.* The S-100 protein was prepared from ox brain according to the procedure of Moore (1965). The purity was checked by immunodiffusion and disc electrophoresis by the methods of Ornstein (1964) and Davis (1964).

*Immunochromatographic procedure.* For each experiment fifty planarians were pooled and the heads were separated from the bodies with a razor blade immediately behind the auricles, and separately homogenized by hand at 4°C in a glass conical homogenizer (Larrabee et al. 1963) in 100 µl of 10 mM Tris-Cl buffer (pH 7.4) containing phenylmethylsulphonylfluoride (PMSF, Sigma Chemical Co, St. Louis, MO) 0.1 mM to minimize proteolytic degradation (Ballal et al. 1975). The homogenates were diluted with 500 µl of the same buffer and centrifuged at 105,000 x g for 90 min. The supernatants were collected. The planarian supernatants were tested by microcomplement fixation assay as described by Moore and Perez (1966) using anti-ox S-100 specific antisera. By comparison, the S-100 was also measured by the same method in a 105,000 x g supernatant of whole brain of adult rat, previously homogenized (1:10, v/v) in a 10 mM Tris-HCl, 0.1 mM PMSF buffer (pH 7.4). Ox S-100 was used as a standard. Control samples with preimmune rabbit serum or with anti-S-100 absorbed with the antigen were also tested. Sera were inactivated by heat at 56°C for 30 min and were usually diluted 1:400 before the assay. Lyophilized complement was a product of Istituto Sieroterapico Milanese Belfanti, Milano, Italy; hemolysin and sheep red cells were purchased from the Sclavo Company, Siena, Italy. Samples with complement alone and with complement and antiserum were assayed to test the anticomplementary activity of tissue extracts and antiserum. All values were corrected for anticomplementary activity, when present, and were expressed in terms of ox S-100 equivalents.

*Immunocytochemical procedure.* Planarian heads were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 3 h at 4°C as described by Greminini and Domenici (1974), and rinsed overnight in 0.1 M phosphate buffer at 4°C. Slices (80 µm thick) were chopped on a Sorvall TC-2 tissue sectioner and processed for the immunoperoxidase reaction using the unlabeled antibody PAP method (Sternberger et al. 1970). Dilution of reagents in 0.1 M phosphate buffered saline (PBS) (pH 7.4) and duration of reaction were as follows: anti-ox S-100 (1:500), or preimmune rabbit serum (1:500), or anti-ox S-100 exhaustively absorbed with S-100 antigen (1:500), for 1 h; sheep anti-rabbit IgG (1:300) for 1 h; PAP complex (1:100) for 1 h; 3,3'-diaminobenzidine 4 HCl (DAB) (Sigma Chemical Co., St. Louis, MO) in 0.05 M Tris-HCl buffer (pH 7.6) (0.4 mg/ml), containing 0.05% H2O2 for 15 min. At the end of each