



Figure 1. Normal chromosomal complement (7II).

Figure 2. A trisomic cell (6II + 1III, arrow).

$5 \cdot 10^{-5}$ M, $5 \cdot 10^{-6}$ M, 10^{-6} M and 10^{-7} M. Seeds soaked in distilled water provided a negative control and treatment with maleic hydrazide (MH) at 10^{-4} and 10^{-5} M concentrations provided the positive control. Maleic hydrazide has been established as clastogenic in different plant and animal species³. 150 seeds were used in each treatment. Pollen mother cells from test plants were stained with acetocarmine for the chromosomal studies.

Seeds treated with a 10^{-6} M concentration of the platinum compound yielded 4 plants with trisomics and those treated

with 10^{-5} M MH yielded 2 trisomic plants (table). The other concentrations and the control plants did not yield any trisomics.

All the trisomic plants had certain common characteristics, i.e. small height, late tillering, large number of tillers, and partial sterility. These plants could not be distinguished from each other as to a particular trisomic on the basis of criteria suggested by Gill et al.². Trisomic cells can be distinguished by the presence of an extra chromosome (fig. 2) from the normal cells (fig. 1).

- 1 We gratefully acknowledge the National Science Foundation grant No. DEB76-04150 to H. Hellmers for the Duke University Phytotron where a part of this study was conducted.
- 2 Gill, B.S., Virmani, S.S., and Minocha, J.L., *Can. J. Genet.* 12 (1970) 474.
- 3 Haley, T.J., *J. Tox. environm. Health* 2 (1977) 1085.
- 4 Khush, G.S., *Cytogenetics of aneuploids*. Academic Press, New York/London 1973.
- 5 Lecointe, P., Macquet, J.P., Butour, J.L., and Paoletti, C., *Mutations Res.* 48 (1977) 139.

- 6 O'Neill, J.P., Couch, D.B., Machanoff, R., San Sebastian, J.R., Brimmer, P.A., and Hsie, A.W., *Mutation Res.* 45 (1977) 107.
- 7 Turnbull, D., Popescu, N.C., Di Paolo, J.A., and Myhr, B.C., *Mutation Res.* 66 (1979) 267.
- 8 Valencia, R., United States Environmental Protection Agency, Contract No. 68-01-2474; final report, 1977.

0014-4754/83/010083-02\$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1983

Influence of breeding temperature on an antibody response (anti SRBC) in a teleostean fish, *Dicentrarchus labrax*

L. Cordier-Perroy, P. Deschaux and G. Peres¹

Université de Limoges, UER des Sciences, Laboratoire d'Immunophysiologie générale et comparée, 123, rue Albert Thomas, F-87060 Limoges Cedex (France), and Laboratoire maritime de Physiologie de Tamaris sur Mer, F-83500 La Seyne sur Mer (France), April 1, 1982

Summary. The effect of temperature on T helper lymphocytes was studied in the spleen of a fish (*Dicentrarchus labrax*). The cooperation phenomenon was demonstrated, with a maximal activity when animals were maintained at 18 °C.

The cellular immune system of fish has been analyzed in fundamental and applied studies. The aim of this work has been directed towards applying newly acquired basic knowledge to understanding the mechanism involved in protecting fish against microbial infection. This paper reports our initial findings on the cellular cooperation of lymphocytes with T-lymphocytes B in a teleostean fish 'le loup de mer' (*Dicentrarchus labrax*) bred at different temperature. No other reports concerning this fish have been found.

Materials and methods. Animals. The fishes, 1-year-old, were obtained from the DEVA-Sud fisheries (CNEXO-Palavas les Flots, France) and bred in our maritime laboratory (Laboratoire maritime de Physiologie, Tamaris sur Mer, France). Animals were kept in aquaria with aerated water at 13, 18 and 21 °C. They were acclimatized for 4 weeks at each temperature before antigen injection (30 animals for each temperature). Experiments were carried out in January and February. Antigen and immunization. Sheep red blood cells (SRBC)

were obtained from BioMérieux Laboratories (France). The cells were washed 3 times with phosphate buffered saline (PBS, pH 7.2) before use.

After 4 weeks of acclimatization at each temperature, 25 animals of each lot were immunized by an intracoelomic injection of $2.3 \cdot 10^6$ SRBC in 0.2 ml, 0.5 M NaCl; 5 animals received 0.2 ml 0.5 M NaCl (controls).

Plaque forming cell assay. 14 days after immunization, plaque forming cells (PFC) were detected using the method described by Jerne et al.². *Dicentrarchus labrax* serum was used as complement source. After a 1st incubation (2.5 h, 25 °C) of the plaques³, complement was added and plaques developed at 25 °C for 2 h. Viability of lymphoid cells was determined with the dye exclusion assay (0.2% trypan blue in PBS).

Results and discussion. Our results are summarized in the table. It can be seen that a peak of the primary response is observed when animals were bred at 18 °C. In a number of teleostean fishes it has been demonstrated that temperature affected antibody synthesis and secretion; a low tem-

perature could decrease these functions. Our results show the effect of temperature on the SRBC response in *Dicentrarchus labrax* with an optimum at 18 °C⁴⁻⁶. In earlier work we have shown the presence of NK cells in this fish, and the activity of these cells was temperature-dependent⁷. This previous work proved the importance of breeding temperature on cellular immunity (T cell dependent). Moreover, because lymphocytes of fishes can be cultivated at low temperatures, we could study, in parallel, the immune response level and the structural and biochemical modifications of these cells which could explain this cooperation phenomenon.

Results for 10⁶ recovered lymphocytes

Breeding temperature	13 °C	18 °C	21 °C
PFC mean ± Sm for each temperature		← * →	← * →
	33.04 ± 5.14	217.3 ± 18.8	39.6 ± 4.8
Number of assays	20	22	19

← * → Significant difference between results for 2 temperature (Student's t-test: p < 0.001).

- 1 Financial support for these studies was provided in part from CNEXO. The technical assistance provided by A. Rigal is gratefully acknowledged.
- 2 Jerne, N.K., Henry, K., Nordin, A.A., Fuji, H., Koros, A.M., and Lefkowitz, Transplant Rev. 18 (1974) 130.
- 3 Rijkers, G.T., and Van Muis Winkel, W.B., Immunology 41 (1980) 91.
- 4 Avtalion, R.R., Immunology 17, (1969) 927.
- 5 Cone, E., and Marcalonis, J.J., Immunology 108 (1972) 952.
- 6 Paterson, W.D., and Fryer, J.L., J. Fish. Res. Board Can., 31 (1974) 1743.
- 7 Deschaux, P., Cordier-Perroy, L., and Pérès, G., Immun. Microbiol. Infect. (1981).

0014-4754/83/010084-02\$1.50 + 0.20/0
©Birkhäuser Verlag Basel, 1983

Studies on the Dd antigen-antibody system. III. Investigations on antigen Dd-reactivity in families

H. Kaur¹ and P.K. Shrivastava²

Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia (Pennsylvania 19111, USA), and Department of Anthropology, Sagar University, Sagar-470003 (India), February 2, 1981

Summary. This paper reports the results of observations on antigen Dd-reactivity in families, age of onset of the trait, and its distribution with respect to sex.

Antigen Dd, present in certain samples of human dandruff, has the ability to form precipitate with selected human sera. The frequency of antigen Dd-reactors varies widely in different populations³⁻⁶. The antigen is heat stable and prolonged storage at low temperatures does not seem to affect it.

The antibodies formed in response to antigen Dd appear to be a permanent characteristic of an individual and this indicated to us that antigen Dd-reactivity may have a genetic background. The present investigations were thus carried out to explore this possibility.

Materials and methods. Venous blood samples were obtained aseptically from 350 individuals belonging to 60 families from the major castes of Punjab. The families represented, in most cases, 3 generations. For certain comparisons, we had available with us, information on antigen

Dd-reactivity in 300 adult Punjabi blood donors from a previous study (2nd paper in this series). The methods of analysis and other technical details were the same as described earlier (1st paper in this series).

Results and discussion. We have observed that human sera which react with antigen Dd, retain this ability for considerable lengths of time. Indeed, we have reason to believe that antibodies to antigen Dd may be a permanent feature in a reactor individual because all our sera found to be reactors in 1976 have remained reactors to date. Furthermore, an individual found to be a reactor by Shrivastava³ in 1972, continues still to have antibodies to antigen Dd. These observations suggest that genetic factors may be involved in the immune response against antigen Dd. We started investigations in this direction by first analyzing 50 cord sera of Punjabi origin for the presence of antibodies against

Table 1. The frequency of antigen Dd-reactors and nonreactors in families with at least one reactor

Sample	Dd-reactor		Dd-nonreactor		Total offspring
	Number	%	Number	%	
Families with excess of Dd-reactors (n = 7)	25	65.79	13	34.21	38
Families with excess of Dd-nonreactors (n = 18)	32	27.83	83	72.17	115
Families with reactors and nonreactors in equal proportion (n = 6)	13	50.00	13	50.00	26
Total (n = 31)	70	39.11	109	60.89	179