Modulation of antibody synthesis by an anti-tumour alga[1]

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Summary. An unicellular alga, *Chlorella pyrenoidosa,* which had been reported to protect C_3H mice against sarcoma BP8, is shown, when injected in Freund's incomplete adjuvant, to modulate the antibody synthesis induced by immunization with a hapten-carrier complex. *C. pyrenoidosa* appeared to be able to initiate an antigenic competition between hapten and carrier determinants of the antigen molecule during antibody synthesis, and thus it could be speculated that *C. pyrenoidosa* modulates the immune response at the macrophage level.

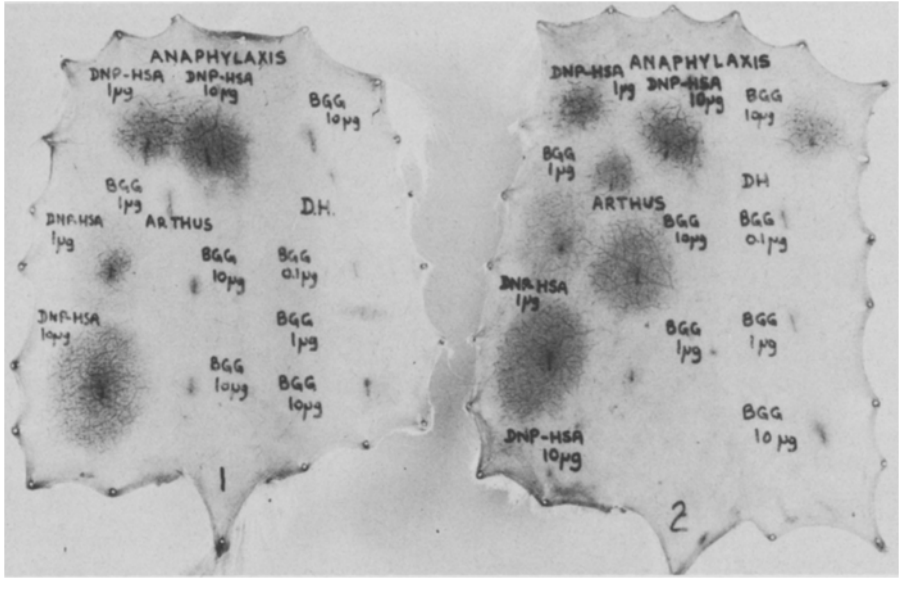
Bacteria[3,4] and fungi[5] are known to inhibit tumour growth possibly by stimulation of immune defences of hosts[6]. Recently, Vermeil and Morin[7] have shown that unicellular algae, *Chlorella pyrenoidosa* could effectively protect C_3H mice against sarcoma BP8. The enhanced resistance to neoplasia induced by *C. pyrenoidosa* could be due to its stimulating influence on immune processes. In this work, we have studied the effetcs of *C. pyrenoidosa* on the responses to a hapten-carrier complex which normally induces antibody synthesis to the hapten and delayed hypersensitivity (DH) reactions to the carrier[8]. The hapten-carrier model has been previously used to dissociate T and B cell functions[9]. The T cells are directly involved in DH to the carrier[10], while cooperation between T and B cells is needed for antibody synthesis to the hapten[11]. When injected i.v., *C. pyrenoidosa* did not modify the response to the hapten-carrier conjugate, while injected s.c. mixed with antigen in Freund's incomplete adjuvant (FIA) they appeared to modulate anaphylactic antibody synthesis depressing antibody synthesis to the hapten and promoting antibody synthesis to the carrier.

Hartley guinea-pigs, immunized i.v. with 4 mg of dinitrophenylated bovine gamma globulins ($DNP_{48}BGG$) on day 0, were injected i.v. with 5×10^6 *C. pyrenoidosa* at days -30, -15 and 0 or with 5×10^8 *C. pyrenoidosa* at days -9, 0 and $+3$. (*C. pyrenoidosa:* strain 211/8b from the collection of Göttingen University, FRG).

In other experiments, animals were injected s.c., in the hind footpads, with various doses of *C. pyrenoidosa* (4×10^8, 4×10^7 and 4×10^6) emulsified in 0.1 ml of FIA with 50 µg of $DNP_{48}BGG$. Skin tests were performed in groups of 5 animals at days 8 and 12 after immunization. In order to test the different types of hypersensitivities, intradermal injections of antigens were done according to the schedule described by Voisin and Toullet[12].

For testing delayed hypersensitivity, 0.1, 1 and 10 µg of BGG in 0.1 ml of saline were injected 24 h before killing the animals. Arthus and anaphylaxis types of hypersensitivities were tested by injecting 1 and 10 µg of $DNP_{43}HSA$ and of BGG, 140 min and 10 min before killing the animals. Evans blue (0.24 ml of a 0.5% solution per 100 g b.wt) was injected i.v. 2 h before killing the animals (20 min after the i.d. injections for Arthus hypersensitivity).

When injected i.v., whatever the doses used or the interval of time chosen between treatment and i.v. immunization, *C. pyrenoidosa* neither depressed nor enhanced any of the responses induced by immunization with the hapten-carrier complex. After s.c. injections of *C. pyrenoidosa* in FIA, guinea-pigs exhibited a strong but transient inflammation of the foot-pads depending on the dose injected. As shown in the table, animals treated with the higher doses of *C. pyrenoidosa* showed depressed anaphylactic reactions at day 8 and at day 12. Furthermore, animals injected with 4×10^6 or 4×10^7 *C. pyrenoidosa* exhibited anaphylactic reactions to the carrier, whereas controls and animals injected with 4×10^8 *C. pyrenoidosa* exhibited no reaction to the carrier (figure). At day 16, anaphylactic reactions of treated animals were similar to those observed in controls. Kinetics of Arthus reactions and of hemagglutinating antibody levels, in all groups, were similar to those of anaphylactic reactions, and for this reason are not shown here. No DH reactions could be elicited by treatment with *C. pyrenoidosa.*



Anaphylactic reactions, at day 12, induced in guinea-pigs by immunization with 50 µg of $DNP_{48}BGG$ in 0.1 ml of FIA alone (1) or in 0.1 ml of FIA containing 4×10^7 *C. pyrenoidosa* (2).

Anaphylactic reactions in groups of 5 guinea-pigs immunized s.c. with 50 µg of $DNP_{48}BGG$ in FIA

No. of *C. pyrenoidosa* emulsified with the antigen in FIA	Anaphylactic reactions to the hapten on day		Anaphylactic reactions to the carrier on day		Foot pads inflammation on day	
	8	12	8	12	8	12
0	13.0±1.7	15.2±1.0	0	0	+	+
4×10^6	12.0±1.5	14.4±1.4	0	12.6±2.9	+	+
4×10^7	9.6±1.2 (1)	12.6±0.8 (3)	0	12.4±1.9	+++	++
4×10^8	11.0±1.4 (2)	11.8±1.7 (3)	0	0	++++	+++

The results are expressed as Evans blue extravasation diameters in mm (±SE) after i.d. injections of 10 µg of antigen. Degree of foot pads inflammation are quoted from + to ++++. (1) $p<0.05$. (2) $0.1>p>0.05$. (3) $p<0.02$.

C. pyrenoidosa, which have been reported to protect C_3H mice against sarcoma BP8, appeared, when injected in FIA, to modulate the antibody synthesis induced by immunization with a hapten-carrier complex. Their effects were transient, observed only at the beginning of the response and could not be directly correlated with the inflammation at the site of injection. On the other hand, the effects of *C. pyrenoidosa* depended on the dose injected. A heavy dose led to a depression of the antibody synthesis while a light dose led to a very small depression of reactions to the hapten but to the induction of antibody synthesis to the carrier. A medium dose of *C. pyrenoidosa* appeared to depress antibody synthesis to the hapten and to induce antibody synthesis to the carrier. The possible immune response to algal antigens could not account for these results. It could be assumed that *C. pyrenoidosa* initiate an antigenic competition between hapten and carrier determinants in antibody synthesis without concomitent variation of DH during the early response to a hapten-carrier complex. If this is true, as antigenic competition between hapten and carrier moieties of the antigen molecule has been previously shown to occur at the macrophage level[13], it could be speculated that *C. pyrenoidosa* modulates antibody synthesis through the macrophage.

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Pineal N-acetyltransferase depression in rats exposed to heat

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Summary. Exposure of adult male rats to increased temperature of 33±1 °C for 3 and 10 days brought about decreases in pineal N-acetyltransferase activity. These and previous findings of pineal HIOMT inhibition under similar conditions support the postulation of a possible thermoregulatory role for the pineal gland.

It has been established that the mammalian pineal is a neuroendocrine gland transducing external environmental stimuli into certain hormonal and behavioural processes. Among the external stimuli influencing the metabolic and hormonal activity of the pineal gland, the effect of environmental lighting has been most thoroughly investigated. Exposure to continuous light, or to long daily photoperiods, was found to decrease the weight[1], protein and RNA content[2] of the rat pineal and to inhibit strongly the activity of N-acetyltransferase (NAT)[3], and of hydroxyindole-O-methyltransferase (HIOMT)[4] responsible for the biosynthesis of the pineal specific hormone melatonin. Little is known about the relationship of the pineal to environmental stimuli other than light, such as temperature, sound, etc. Brief and prolonged exposure of rats to low temperature produced pineal hypertrophy and increased metabolic activity[5,6], while high temperature decreased pineal contents of protein and RNA[7] as well as HIOMT activity[8]. Since NAT activity was found to be highly sensitive - much more so than HIOMT - to changes in environmental lighting, and as NAT is the primary enzyme in the synthesis of melatonin, we decided to investigate the effect of high environmental temperature on this pineal enzyme.

Materials and methods. Male rats weighing 160-180 g were divided into 3 groups of 6-8 animals each and housed 3 or 4 to a cage. 2 groups were exposed to a constant heat of 33±1 °C, one for 3 and the other for 10 days; the 3rd group, which served as control, was kept at a temperature of 24±1 °C. Light was switched on at 06.00 h and off at 18.00 h each day. After exposure to their respective thermoenvironments for 3 or 10 days, the rats were decapitated either between 22.30 h and 23.00 h or between 10.30 h and