Showing number of gestating mice irradiated with 50 R gamma radiation in presence or absence of MPG and average litter size born

Day of irradiation (postconception)	Mode of treatment	No. of animals used	No. of young born	Average litter size	Young ones born (%) Male	Female	Mortality after birth (%)
	Normal	52	448	8.6	54.0	46.0	4.0
14.25	Control (irradiation)	10	88	8.8	48.8	51.2	2.0
	Experimental (irradiation + MPG)	8	70	8.7	52.8	47.2	Nil
16.25	Control	7	56	8.0	55.4	44.6	Nil
	Experimental	9	77	8.5	51.9	48.1	2.0
18.25	Control	12	96	8.0	47.9	52.1	Nil
	Experimental	8	67	8.3	54.0	46.0	Nil

sence of a 2nd phase of weight loss in female offspring may be due to the low dose used in this case. Females are reported to be less sensitive to radiation than males[15].

The chemical protector, cysteamine has been reported to protect the mouse fetus against irradiation[7]. MPG protected significantly the young ones against body weight loss after exposure to higher doses and also averted a 2nd phase of weight loss[10,11]. In the present study, animals in the experimental group (MPG treated) showed less weight reduction in the early intervals and almost complete recovery after 2 weeks. It appears that MPG arrested the mitotic activity of the cells[16] by reducing the mitotic-linked cell death, and protected against the initial weight loss. The increase in rate of mitotic activity following an initial stress in protected offspring may explain the absence of a 2nd weight loss due to the dose used in the present experiment.

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Normal immunosuppressive protein: inhibitory effect on hemagglutinin and plaque formation as well as B cell transformation by Epstein-Barr virus

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Summary. Injections of normal immunosuppressive protein (Nip) cause a significant decrease of hemagglutinin formation and plaque formation in rats immunized with sheep red blood cells. Nip added in vitro to human unbilical cord lymphocytes inhibits the B cell transformation caused by Epstein-Barr virus.

The immune reactivity is the result of a delicate balance between the stimulatory activity and the inhibitory activity triggered by the antigen. The modulation of this process is at present only partially understood. For several decades the enhancing part of the immune reaction has been widely studied and extensive knowledge has been accumulated, but the importance of suppressor factors and suppressor cells has been realized only in the last decade and our knowledge in this area is less extensive.

One of the suppressive factors that has been extensively studied in our laboratory is normal immunosuppresive protein (Nip)[2]. It is isolated from human blood or amniotic fluid[3]. By elution experiments with mannose from concavalin A-sepharose columns it has been shown to be a glycoprotein or glycopeptide, which is resistant to boiling and shows a positive PAS staining reaction[4].

The molecular weight of Nip, as determined by SDS polyacrylamide gel electrophoresis on the one hand and inability to leak through dialysis membranes with pore size of less than 10,000 on the other is about 15,000 daltons. It suppresses all lymphocyte activity whether expressed as B cell activity or T cell activity[5]. It also inhibits NK cell activity[6] and prevents the generation of suppressor cells[7]. At the same time Nip has no effect on macrophages or myeloid cells[8]. The binding of Nip to lymphocytes is reversible. It prevents DNA and RNA synthesis in the lymphocytes, is not toxic to the cells, and when it is washed away the cells regain their ability to carry out DNA and RNA synthesis after an interval for regeneration is allowed[3]. Nip is not species specific and the material prepared either from human plasma or human amniotic fluid shows similar suppressive activity on both human and

murine lymphocytes[8]. The trial of Nip in various immunological tests reveals that in vivo it causes the prolongation of skin allograft and xenograft survival times[2]; it also decreases the resistance of mice to infection with group A streptococcus by inhibiting antistreptococcus antibody formation and depressing bactericidal activity[9]. In in vitro tests Nip inhibits phytohemagglutinin-induced, concavalin A and lipopolysaccharide mitogenic responses as well as antibody mediated cytotoxicity[5]. It also prevents the generation of primary cytotoxic effector lymphocytes against allogeneic tumor cells[7]. The finding of elevated Nip levels in patients with malignant diseases suggests that Nip could lead to a reduction in the immune surveillance potential of the host when present in high concentrations[10]. Indeed, the injection of mice with Nip enhanced the growth of syngeneic methylcholantrene-induced tumors, even in tumor cell doses normally rejected by untreated mice[11].

The present report describes the in vivo activity of Nip in reducing the immune response towards sheep red blood cells in rats as well as the in vitro inhibition of B cell transformation of human cord blood lymphocytes by Epstein-Barr virus.

Materials and methods. 0.2 ml Nip isolated as previously described[3] was injected i.v. into rats in concentrations of 5–15 mg protein/ml. Injections of 2.5×10^8 SRBC were given i.p. 30 min, 1, 2 and 3 days before the injection of Nip, or 1 day after the injection of Nip. In control experiments, similar concentrations of human serum albumin were injected instead of Nip. All animals were sacrificed 6 days after the intraperitoneal injection of SRBC. The hemagglutinin titer was determined by the double serial dilution method and the Jerne plaque forming test was performed with the spleen cells according to the modifications of Wortis et al.[12]. The direct and indirect number of PF cells was calculated as the number of plaques per 10^6 spleen cells. The influence of Nip on B cell transformation by Epstein-Barr virus (EBV) was examined by a method suggested by Jondal and Klein[13]. 1×10^6 cord blood cells in quantities of 0.5 ml were incubated with an infective dose of EBV. Three different concentrations of Nip were than added and weekly observations were made on the viability of the cells as well as on the formation of clumps indicating a typical cell line. In control experiments the cells incubated with EBV alone became cell lines 3 weeks after the beginning of the experiment.

Results. The most significant reduction of both the hemagglutinin titer and the number of plaque forming cells was observed when Nip was injected 30 min before the antigen. The injection of Nip, even in large amounts, 3 days prior to the antigen was ineffective; similarly, Nip injected 1 day after the antigen did not have any inhibitory effect. Figure 1 gives some typical results of such an experiment. Every result in table is the mean value for 10 rats. Figure 2 represents the inhibition of 7S and 19S hemagglutinins and plaques according to the quantity of Nip injected. Every column is the mean for 10 rats. The influence of Nip on B cell line transformation by Epstein-Barr virus was very clear. At the low Nip concentration (0.25 mg protein/ml) all 5 tubes showed cell line formation but the appearance was delayed to 5 weeks. In the higher Nip concentration a typical dose response curve was observed.

Discussion. The immunosuppressive effect of Nip is clearly shown by its ability to suppress hemagglutinin formation and B cell transformation. Several other factors found in

	Appearance of line (weeks)	No. of tubes in which line appeared
Cells alone	-	0/5
Cells + EBV	3	5/5
Cells + EBV + Nip (0.25 mg/ml)	5	5/5
Cells + EBV + Nip (0.5 mg/ml)	5-6	2/5
Cells + EBV + Nip (1 mg/ml)	-	0/5

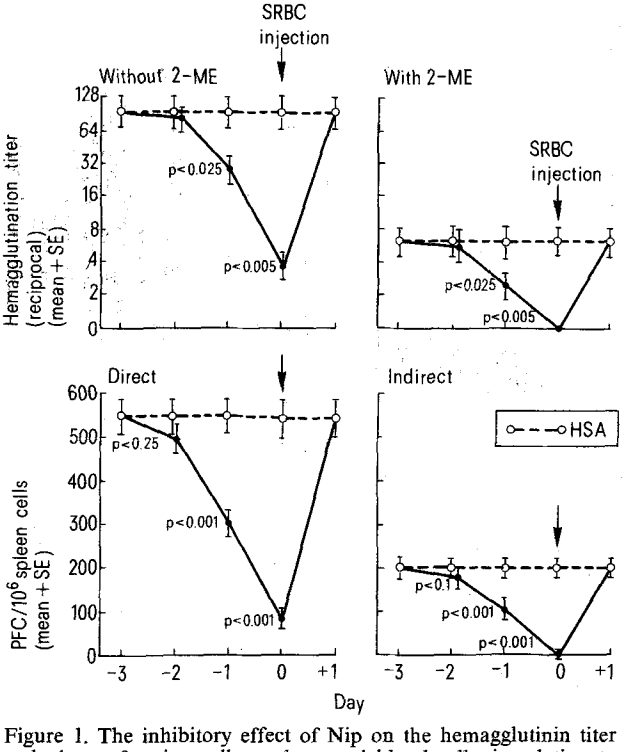

Figure 1. The inhibitory effect of Nip on the hemagglutinin titer and plaque forming cells to sheep red blood cells, in relation to time of immunization.

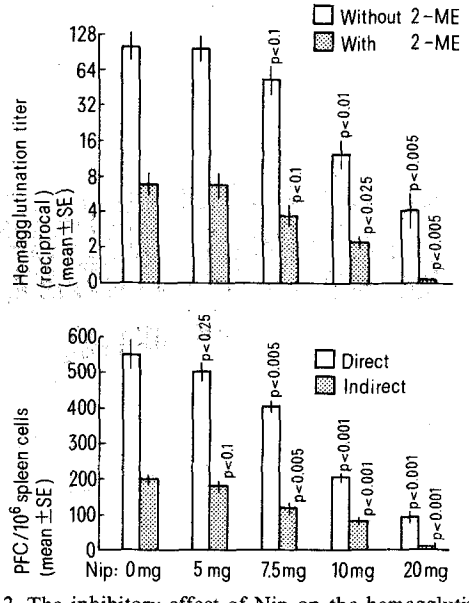

Figure 2. The inhibitory effect of Nip on the hemagglutinin titer, and plaque forming cells to sheep red blood cells in relation to quantity of Nip injected.

mammalian blood have proved to cause immunosuppression in various in vivo and in vitro tests, but Nip has been shown to be different, demonstrating no crossreactivity with the following; α fetoprotein, as determined by radioimmunoassay, pregnancy associated α_2 macroglobulins, β_1 glycoprotein, C reactive protein, as determined by immunoelectrophoresis. The same was true of fibrinogen degradation products and several others that were extracted from liver tissue, lymphatic tissue, macrophages, thymus and tumors, as determined by absorption of anti-Nip antiserum and the quantitative examination of this serum against Nip in the Elisa method. In many respects Nip is similar to immunoregulatory α globulin (IRA)[14] but differs from it in its strong immunosuppressive activity on B cells (immunological cross reactivity, however, has not been tested).

At present it is not known where and how Nip is produced nor do we know what causes elevation of Nip levels. In spite of these uncertainties it is clear that Nip and/or similar serum factors praticipate in the control mechanisms of the immune response.

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The effect of continuously cooling the hypothalamic preoptic area on antibody titre in the rat

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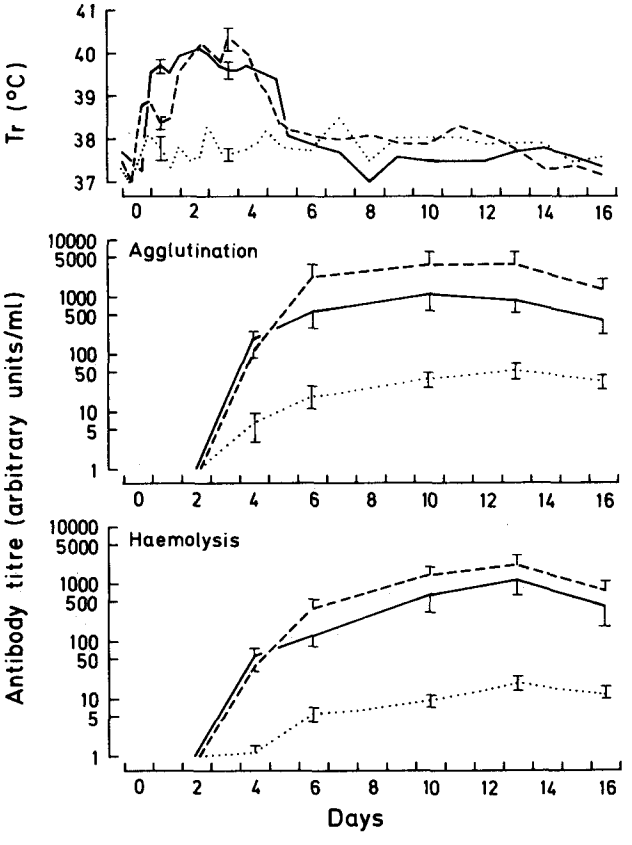
Summary. Continuous cooling of the hypothalamic preoptic area for 5 days, as well as sublethal infection with *Salmonella enteritidis*, increased the titre of antibodies against sheep erythrocytes, suggesting that the febrile response stimulates the humoral immune response.

It has long been thought that fever may stimulate the immune system[2], though passive hyperthermia often inhibits it[3,4]. Some support for this view was recently obtained in experiments in which intermittent fever, either induced by an endogenous pyrogen[5] or simulated by cooling the hypothalamic preoptic area[6], increased the titre of antibodies. Many infectious diseases elicit, however, continuous fever, and this sustained increase in body temperature might harm the immune system. In the present work, therefore, we studied the effect of continuous fever, simulated by cooling the hypothalamic preoptic area, and we show that it also stimulates the humoral immune response.

Methods. During the spring, white male rats weighing about 350 g were maintained at an ambient temperature of 23 °C with natural illumination. Food and water were continuously available. In some of the rats, a thermode was chronically implanted into the preoptic area, and the animals were then fixed to an antirotatory device[7]. 1 week afterwards, these animals were randomly divided into 2 groups, hypothalamus-cooled and control, of 7 animals each. The rest of the animals, all without thermodes, were



Body temperature and titre of antibodies against sheep erythrocytes in 6 hypothalamus-cooled (solid line), 5 infected (broken line) and 13 control animals (dotted line). At noon on day 0, all animals were immunized with sheep erythrocytes, while those of the infected group were additionally injected with live *S. enteritidis* (0.0002 mg bacterial dry weight). The thermodes of the animals of the hypothalamus-cooled group were perfused with cold water from day 0 at 18.00 h until day 5 at 10.00 h. The vertical lines are SE's.