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IMMUNE SYSTEM MODULATION AND ITS EFFECT ON THE BLOOD PRESSURE OF THE SPONTANEOUSLY HYPERTENSIVE MALE AND FEMALE RAT

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Spontaneously hypertensive rats (SHR) demonstrate a consistently lower immune response to Con A as compared to Wistar Kyoto (W/K). Using anti-thymocyte serum, T and B cell responses of SHR drop considerably while blood pressures decrease to normal levels. Levamisole treatment has little or no effect on mitogen responses, but depresses blood pressure of SHR. It is hypothesised that hypertension in SHR is autoimmune in nature.

The spontaneously hypertensive rat (SHR)[1] was originally described by Okamoto and Aoki (1) as a strain that develops an idiopathic hypertension at about three months of age. The hypertensive state is greater in males than in females and persists with typical human-type kidney and cardiovascular complications until death of the animal at about 24 months of age.

SHR have been shown to have an enlarged thymus (2). In a genetically hypertensive mouse strain (NZB/Cr) removal of the thymus very early in life or prenatally resulted in failure to develop hypertension (3). Takeichi et al. (4) have reported that guinea pig T cell rosettes of the SHR declined with age and that T and B cell depressed responses were more evident among older age groups.

This study was undertaken to evaluate the immune response to mitogens of male and female SHR and to compare these with the W/K controls. Since

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[1]Abbreviations: SHR, spontaneously hypertensive rat; Pg, prostaglandin; RIA, radioimmune assay; ATS, anti-thymocyte serum; SC, subcutaneously

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prostaglandins of the E series have been shown to be immunosuppressive (5,6), spleen supernatants of the SHR and W/K were examined for PgE content by RIA. Lastly, attempts were made to alter blood pressures of the SHR by means of the immune modulating agents anti-thymocyte serum (ATS) and Levamisole.

MATERIALS AND METHODS

Male and female SHR and W/K rats were purchased from Taconic Farms Inc. at 4 weeks of age. The animals were housed in hanging cages, kept on a 12 hr light/dark cycle, and fed Purina rat chow and tap water ad libidum. Body weights and blood pressure measurements (7) were monitored weekly, beginning 8:30 AM. A total of 56 treated and untreated SHR were used with 30 W/K controls.

Animals were sacrificed by cervical dislocation. Each spleen was removed asceptically and placed into a sterile 60 x 15 mm petri dish (Falcon) containing 5 ml complete RPMI medium 1640. Complete RPMI medium consisted of RPMI to which was added 25 mM Hepes buffer, 200 mM glutamine (Microbiol. Assoc.), 10% heat inactivated fetal bovine serum (Flow Laboratories), 0.1% gentamicin (Schering), or 1% penicillin/streptomycin solution containing 10,000 units/ml (Gibco). Individual spleen cells were prepared as previously described (8). Mitogen assays were performed in triplicate by the method of Thurman and Goldstein (9).

A volume of 0.2 ml of rabbit anti-rat thymocyte serum (Microbiol. Assoc.) was administered SC daily for 14 days to 9 week old male and female SHR. An equal number of male and female SHR were injected with 0.2 ml sterile physiological saline for 14 days. Animals were sacrificed 14 days after the final injection of ATS or saline.

Levamisole (Sigma) was prepared daily in sterile saline and was administered SC at a dose of 5 mg/kg body weight for 14 days to 9 week old male and female SHR. An equal number of male and female SHR were injected with 0.5 ml sterile saline for 14 days. Animals were sacrificed 4 days after the last Levamisole or saline injection.

Two ml of the spleen cell preparation (5×10^6 cells/ml) in complete RPMI medium were placed in 4 individual wells of 24 flat bottomed Linbro plates (Flow Laboratories). The cells were incubated at 37°C for 24 hr in a 5% CO_2 humidified atmosphere. One ml of supernatant was placed in a polypropylene tube following centrifugation for 10 min at 1500 rpm. A volume of 0.1 ml of 0.3 M citric acid was added for preservation of Pg. Extraction of Pgs from spleen cells were performed by modification of the method of Poleshuck et al. (10). RIA was performed by the method of Clinical Assay, Inc. using anti-Pg B_1 (Analytic Assoc.).

RESULTS

The blood pressures of untreated male SHR were markedly elevated over those of male W/K starting at approximately 5 weeks of age. At 12 weeks of age, the blood pressure of male SHR reached 210 mm Hg and remained at that

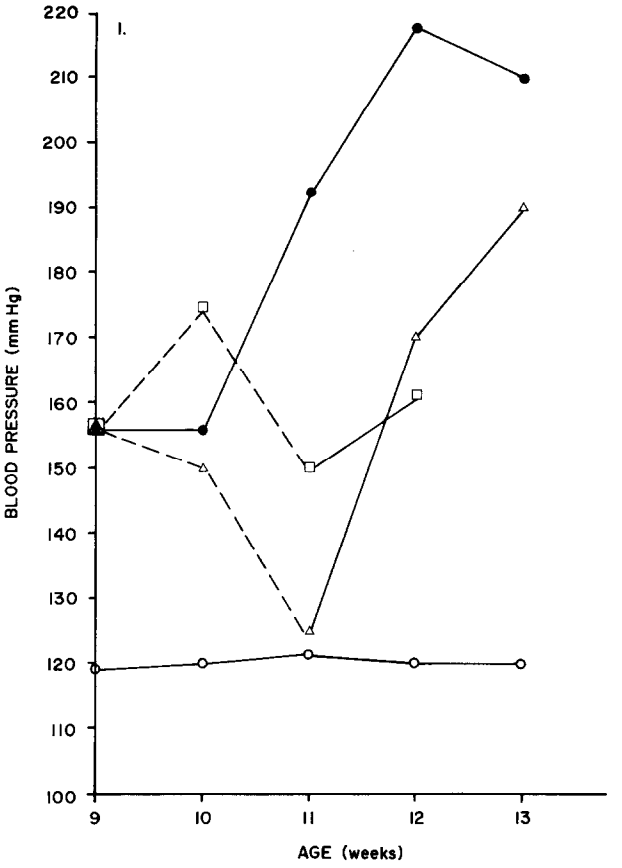

Figure 1. Mean blood pressures of male W/K (○), untreated SHR (●), Levamisole treated SHR (□), and ATS treated SHR (△). Treatment period (- - -). Standard deviations at all points shown were ± 5-10 mm Hg. The results for sham injected rats were similar to those shown for untreated rats.

level until the end of the experiment at 17 weeks (Fig. 1). SHR female blood pressures were similarly higher than female W/K and continued to rise to 180 mm Hg by the 17th week (Fig. 2). W/K rats did not show this sex differential and maintained normotensive pressures throughout the experimental period (115 mm Hg, Fig. 1 & 2).

Administration of ATS to male SHR caused an initial small decrease in blood pressure followed by a rapid decline of 60 mm Hg (as compared to sham-injected and untreated SHR) and approached the level of the W/K male (Fig. 1). Termination of ATS administration led to an immediate increase in

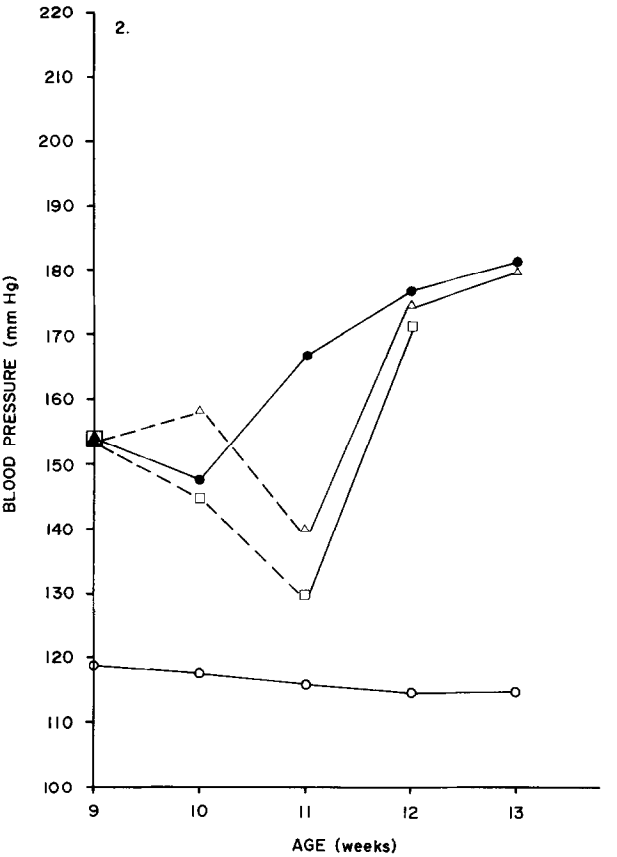

Figure 2. Mean blood pressures of female W/K (○), untreated SHR (●), Levamisole treated SHR (□), and ATS treated SHR (△). Treatment period (- - -). Standard deviations at all data points were ± 5-10 mm Hg.

blood pressure which at 13 weeks of age was 20 mm Hg less than the untreated or sham injected SHR.

Similarly, in ATS treated females, the blood pressure rose after one week of administration and was then depressed by the second week to about 25 mm Hg less than the control SHR. Female SHR blood pressures returned to the level of the control SHR upon termination of ATS treatment.

Male SHR blood pressure increased after the first week of Levamisole treatment and then declined 30 mm Hg as compared to control SHR. Termination of Levamisole resulted in a small increase in blood pressure over the four day period prior to sacrifice. Neither Levamisole nor ATS was as effective in females as in males in decreasing blood pressure to the level of the W/K.

TABLE I

Mitogen stimulation of spleen cells from SHR treated and control rats

		Cells from			
		Controls		Treated SHR's	
Sex	Mitogen	W/K cpm[a]	SHR cpm	Levamisole cpm	ATS cpm
male	none	2900±1200	3600±1400	1500± 300	1100±200
female	none	2500± 800	3500±1100	3000± 400	1000±100
male	Con A	10000±2200	2800±1400	2500± 500	1800±500
female	Con A	31000±9100	12600±4700	8600±5200	3800±700
male	LPS	2500± 400	4300± 600	2700± 300	1500±600
female	LPS	3900± 400	5400± 400	4400±2100	2300±100

[a] mean values ± SD

ATS but not Levamisole depressed the immune responses of male and female SHR spleen cells to stimulation by Con A as compared to sham-treated SHR and W/K. ATS also depressed the immune response of male and female SHR spleen cells to LPS as compared to controls. The response of untreated SHR spleen cells to LPS was higher than that of the cells from W/K rats (Table I). In all experiments, with both strain of rats, immune responsiveness of cells from females was much higher than that of cells from males. Male SHR spleen cells produced about 2 times as much Pg as female SHR spleen cells (Table II). Both the male and female W/K produced similar but lower levels of Pg. Levamisole increased Pg production in female SHR spleen cells as compared to cells from controls but had no effect on male SHR spleen cells. ATS caused a 4 fold decrease in Pg production by male SHR spleen cells and a 1.5 fold decrease in Pg production by female SHR spleen cells (Table II).

DISCUSSION

The concept that the hypertensive state, as seen in the genetic SHR, may be due to an autoimmune etiology similar to that seen in the virus-infected (EMC) diabetic mouse (11) is based on two observations. In both conditions a spontaneous T cell depressed response to mitogens was present

TABLE II

Prostaglandin E production[a]

	Cells from			
	Controls		Treated SHR's	
Sex	W/K cpm	SHR cpm	Levamisole cpm	ATS cpm
male	300±20	510±20	460±30	130±20
female	280±10	250±20	460±20	190±30

[a] Expressed as pg per 5 x 10^6 spleen cells (mean values ± SD)

prior to treatment. The disease symptoms of both were relieved with further reduction of immune reactivity with the use of antiserum to lymphocytes or thymus. Bushard and Rygard (12) also found depressed immune reactivity in streptozotocin-induced mouse diabetes, as well as an inability to induce the diabetes in the athymic nude mouse (13). Like et al. (14) administered ATS to the spontaneously diabetic rat and relieved the diabetic condition. This led to the novel approach used in these experiments, i.e., the attempt to induce a blood pressure reduction by immune modulation.

ATS has been shown to cause depression in T cell responses (15), and, as a consequence, prolong xenograph survival time (16). With the administration of ATS to the SHR the blood pressures of both sexes fell strikingly to within the normal range of the W/K after one week. As expected, the response to Con A was almost completely eliminated. Long term studies on the effect of ATS, thymosin and clondine are now in progress.

Levamisole has been demonstrated to have immunopotentiating effects (17). However, no linear dose response effects have been established with Levamisole in vivo. In addition, the species and strain used, as well as the dosage and time of administration, plays a major role in the response elicited by this drug (18). In the results presented here, blood pressure fell after drug administration. The depressant effect may be due in part to the known sympathetic stimulatory effect of the compound (17). The end re-

sult of this type of adrenergic enhancement may be catecholamine-induced vasodilation. In support of this hypothesis, it was noted that the adrenal size of the Levamisole treated SHR was increased two-fold over that of the untreated rats (data not shown). The ATS treatment did not affect adrenal size.

The observed ATS-induced decreases in Pg levels may indicate a generalized loss in T cell immune function with subsequent decrease in those lymphokines influencing cells such as mononuclear phagocytes which produce Pgs (19).

It may be that the decrease in immune reactivity in the SHR rats, as compared to the W/K strain, provides this hypertensive animal with a life-sustaining mechanism which persists until death at 20 or more months. Spontaneous hypertension in this strain of rat may therefore be classified as a T cell mediated disease with a possible autoimmune etiology. The fact that T cell depression is seen even prior to the treatment with immune depressives indicates that a mechanism exists to homeostatically prevent a life-threatening rise in blood pressure.

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