THYROID GLAND FUNCTION DURING THE SYSTEMIC GRAFT VERSUS HOST REACTION

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KEY WORDS: graft versus host reaction; tri-iodothyronine; thyroxine; thyrotrophin; ^{125}I uptake.

Mechanisms of various kinds of pathology can be studied on a model of the graft versus host reaction (GVHR): autoimmune diseases, lymphoproliferative processes, and immunodeficiency states [1, 6, 7]. The pathogenesis of GVHR itself has not been fully explained. In particular, the role of the endocrine system in the development of GVHR has not been studied. There is evidence in the literature of changes in the adrenals during GVHR [4], but there are no data on thyroid function during GVHR. Meanwhile the thyroid gland is known to participate in immunologic reactions [8]. Thyroid hormones also play an important role in growth and development of the organism [3].

The aims of the present investigation were as follows: to determine the level of thyroid hormones and thyrotrophin (TSH) at various times after induction of GVHR, to study the degree of ^{125}I uptake by thyroid gland tissue at the same times of the GVHR, and to determine correlation between the hormone levels and weight of the gland in the animals and also the body weight of the recipients.

EXPERIMENTAL METHOD

Altogether 200 male hybrid (CBA × C57BL/6)F_1 mice weighing 19-20 g, obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR, were used in the experiments between January and July, 1984. A systemic GVHR was induced by intravenous injection of $60 \cdot 10^6$ spleen cells (SC) from the C57BL/6 parent. Intact F_1 hybrids and F_1 hybrids receiving an intravenous injection of $60 \cdot 10^6$ syngeneic SC served as the controls.

Serum levels of tri-iodothyronine (T_3), thyroxine (T_4), and TSH were determined by radioimmunoassay, using kits of reagents from Byk-Mallinckrodt (West Germany), on the 3rd, 10th, and 24th days after induction of the GVHR. ^{125}I was injected intraperitoneally in a dose of 3-4 μCi/100 g body weight [2]. The animals were killed 1 h after injection of ^{125}I. Radioactivity was measured on a GSB-1 scintillation Gamma-counter. The numerical results were subjected to statistical analysis by Student's test.

TABLE 1. Changes in Weight of Thyroid Gland and Recipients' Body Weight and ^{125}I Uptake by the Thyroid Gland (M ± m)

Experimental conditions	Recipients' body weight, g	Weight of thyroid gland, mm	^{125}I uptake, %
Control	19,2±0,11	3.3±0,17	0,297±0,04
GVHR:			
3rd day	19,0±0,06	3,9±0,23	0,276±0,03
P	>0,05	>0,05	>0,05
10th day	17,0±0,15	3,0±0,19	0,061±0,006
P	<0,001	>0,05	<0,001
24th day	16,3±0,02	2,9±0,28	0,090±0,011
P	<0,001	>0,05	<0,001

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TABLE 2. Serum Levels of Thyroid Hormones and TSH (M ± m)

Hormone	Experimental conditions	Stage of GVHR, days		
		3-rd	10-th	24-th
T_3, mg/100 ml	Intact control (1)	168,9±13,2	—	—
	Syngeneic control (2)	161,0±9,1	147,5±2,97	142,4±8,3
	P_{2-1}	>0,05	>0,05	>0,05
	GVHR (3)	148,8±6,91	90,23±4,01	64,9±8,3
	$P_{3-1,3-2}$	>0,05	<0,001	<0,001
T_4, μg/100 ml	Intact control (1)	4,0±0,7	—	—
	Syngeneic control (2)	3,12±0,34	2,42±0,45	1,86±0,17
	P_{2-1}	>0,05	>0,05	<0,001
	GVHR (3)	2,83±0,41	1,42±0,15	0,728±0,18
	P_{3-1}	>0,05	<0,001	<0,001
	P_{3-2}	>0,05	<0,02	<0,001
TSH, mIU/ml	Intact control (1)	0,460±0,12	—	—
	Syngeneic control (2)	0,380±0,09	0,600±0,23	0,780±0,20
	P_{2-1}	>0,05	>0,05	>0,05
	GVHR (3)	0,300±0,04	0,716±0,19	1,810±0,51
	P_{3-1}	>0,05	>0,05	<0,05
	P_{3-2}	>0,05	>0,05	>0,05

EXPERIMENTAL RESULTS

Uptake of ^{125}I by the thyroid gland of the animals on the 3rd day after intravenous injection of $60 \cdot 10^6$ SC from the C57BL/6 parent did not differ significantly from the control (Table 1). On the 10th day after induction of GVHR, ^{125}I uptake was reduced by 4.9 times compared with the control, and it remained about 3.3 times lower until the end of the 24th day. Incidentally, on the 24th day ^{125}I uptake by the thyroid gland tissue was 1.5 times greater than on the 10th day ($P < 0.05$). The weight of the thyroid gland was unchanged at all times after induction of GVHR.

On the 3rd day after induction of the GVHR, before any decrease in the recipients' body weight, the levels of hormones T_3, T_4 and TSH were indistinguishable from the controls. On the 10th day a significant fall of the T_3 level by 1.8 times and of the T_4 level by 2.5 times was observed. The TSH concentration was unchanged on the 10th day after induction of GVHR, whereas the loss of body weight by the recipients amounted to about 10% of the initial values. On the 24th day after induction of GVHR a more marked fall in the serum thyroid hormone levels was observed than in intact mice: The T_3 level was 2.5 times lower and T_4 5 times lower. Relative to the syngeneic control the T_3 level was twice and the T_4 level 2.5 times as low. The TSH level was sharply raised and was 4 times higher than the control. During this same period the recipients' body weight fell by 13% ($P < 0.001$).

During the development of a systemic GVHR marked inhibition of thyroid function was thus discovered. It is an interesting fact that a fall in the serum T_3 and T_4 concentrations was observed despite no change (10th day after induction of GVHR) or a sharply increased (24th day) TSH concentration. It can be tentatively suggested that the development of GVHR inhibits thyroid function primarily. The fall in the blood levels of the T_3 and T_4 hormones, through the operation of regulating mechanisms, leads to increased production of TSH [5]. However, its increased secretion on the 24th day leads to only a small increase in iodine uptake by the thyroid gland tissue. The blood levels of hormones T_3 and T_4 fell even more during this period. These results are evidence that inhibition of thyroid function during the development of GVHR is not under the influence of the pituitary, but has some other mechanism.

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IMMUNOGENICITY OF ARTIFICIAL ANTIGENS AS A FUNCTION OF NUMBER OF PROTEIN MOLECULES BOUND WITH POLYELECTROLYTES

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Binding of protein and peptide antigens (of serum, bacterial, or viral origin) with artificial polyelectrolytes (PE; polybases and polycarboxylic acids), through complex formation or covalent bonding of the components, can yield highly immunogenic artificial antigens [3, 11, 12]. Correlation has been found between the immunostimulating properties of a series of polyelectrolyte polymer analogs and the immunogenicity of their protein mixtures, on the one hand, and the character of complex formation in these mixtures on the other hand [4]. It has been suggested that the physicochemical basis of manifestation of the immunologic properties of protein–PE complexes probably lies in the ability of free sites of the polymer chain in the composition of the complex (loops, free ends, and so on) to undergo cooperative sorption on the surface of immunocompetent cells [2, 5]. The critical character of the influence of the degree of polymerization of PE on their immunostimulating properties has recently been established [6].

Complex formation between proteins and PE is characterized by a significantly nonhomogenous distribution of protein globules among the polyions adsorbing them [7, 8]. The number of protein molecules per polymer chain depends on the degree of polymerization ($\bar{P}_\eta$) of PE and increases as a linear function of chain length. There is a certain critical value $\bar{P}_{\eta cr}$, above which nonstoichiometric polymer-protein complexes are formed, i.e., the "epitopic" density of the protein in the composition of the polyelectrolyte complex increases. Because of this, the study of the character of dependence of immunogenicity of artificial antigens on the length of the carrier polymer bound with the proteins, and on the immunostimulating activity of free PE, and the study of dependence of immunogenicity on the number of bound protein molecules in the composition of polymer homologs of soluble covalent conjugates are of great importance for the understanding of the mechanisms of immunogenicity of artificial antigens and of the "adjuvant" action of PE.

The aim of the present investigation was to study these problems.

EXPERIMENTAL METHOD

Fractions of polyacrylic acid (PAA) and poly-4-vinylpyridine (PVP) were obtained by known methods [9, 13]. PVP_R were obtained by quaternization of the PVP fractions with bromoacetic acid [7]. The average degree of quaternization was 50%. Narrow fractions of PAA with degrees of polymerization ($\bar{P}_\eta$) of 43 (PAA_1), 170 (PAA_2), 430 (PAA_3), 570 (PAA_4), and 1140 (PAA_5) were chosen as test objects; $\bar{P}_\eta$ for PVP_R was 10^3.

Covalent bonding of PAA and PVP_R was carried out through a stage of activation by carbodi-imide (CDI; from Serva, West Germany) [1, 11].

The immune properties of solutions of the conjugates were studied on C57BL/6 and (CBA × C57BL/6)F_1 hybrid mice weighing 22-24 g, obtained from the Stolbovaya Laboratory Animals Nursery, Academy of Medical Sciences of the USSR. The number of IgM- and IgG-antibody-forming

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