Setting of the experiment

Vial	Inoculated[a]	Diffusion chamber[b]	Control cultures[c] of the vials	of the chamber contents
CM: control, medium	No	No	Negative	
CV: control, valves	No	Yes	Negative	Negative
CB: control, bacteria	Yes	No	Group A streptococcus	
T: test	Yes	Yes	Group A streptococcus	Negative

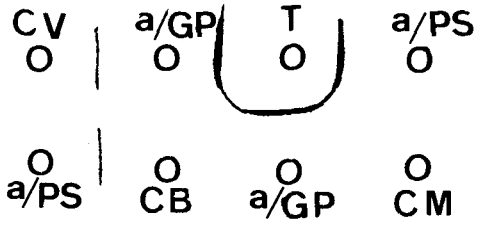
[a] Group A streptococcus, strain A 5205; [b] containing approximately 2 g of human heart valves; [c] on blood agar, after 62 h of incubation of the vials.

were prepared as described[1,3]. Amino acids, amino sugars and sugars in acetone precipitates were identified and estimated as outlined previously.

Results and discussion. The results of immunodiffusion analysis of the acetone precipitates are shown in the figure. No immunoprecipitation was observed with CM precipitates. CB precipitates traced a weak, uneven line with undiluted antipolysaccharide antisera; no line was obtained with 1:2 or 1:4 dilutions of the serum. T precipitates showed strong, forward and constant lines with both antisera, with an identity reaction. These precipitation lines were still present with 1:20 dilutions of the antisera. Results of chromatographic evaluation of amino acids, amino sugars and sugars showed that T extracts, as compared to all other controls, had a marked increase in glucosamine, glycine, proline and hydroxyproline, and a moderate increase in glutamic acid.

These results are in agreement with the preceding observations made on bovine heart valves. The identity reaction of the immunologically active substance(s) is however more complete than with bovine material. The present results do not definitely prove that the immunologically active fraction was extracted from the valve connective tissue, but the results of the chemical determinations favour this hypothesis. CB precipitates showed an increase in cell wall amino acids such as alanine and lysine, which was probably due to spontaneous lysis of some bacteria. In the T precipitate, however, we observed a marked increase in amino acids present only in small amounts in the streptococcal wall and cytoplasm, e.g. glycine, proline, or hydroxyproline[4,5]. On the other hand, these amino acids are components of the connective tissue glycoproteins not extractable by ionic buffers[6] and are found in urea-soluble glycoprotein[3].

These results confirm that the observations previously reported for bovine tissues can provide a basis for hypothesis in human pathology. It is worthy of notice that, although direct immunofluorescence with antistreptococcal antisera was not observed on cardiac tissue sections, it was detected after a prior treatment of the same sections with a proteolytic enzyme[7]. Thus, the unmasking of a 'hidden' tissue determinant by bacterial enzyme(s), leading to an immunological cross-reaction, seems a feasible proposition, specially if one recalls that the original specific lesion of the Aschoff bodies is a peculiar response to a primary injury of the connective tissue[8].



Immunoprecipitation of the acetone-precipitates. CM: control, medium; CB: control, bacteria; CV: control valves; T: test (see table); a/PS: anti-streptococcal polysaccharide anti-serum; a/GP: anti-human glycoprotein anti-serum. Immunodiffusion gel: 1% agarose in 0.17 M NaCl, buffered at pH 6.0 with 0.01 M phosphate buffer 0.01% cadmium acetate.

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Allogeneic effect on induction of thyroglobulin antibodies and thyroid lesions in mice[1]

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Summary. All previous attempts failed to induce thyroglobulin antibodies or thyroid infiltrates in mice immunized with homologous thyroglobulin without adjuvants. However, an allogeneic effect obviated the need of adjuvants for triggering thyroglobulin-reactive lymphocytes to produce thyroglobulin antibodies or thyroid lesions.

Immunization of mice with homologous thyroglobulin emulsified in Freund's complete adjuvant usually induces autoimmune thyroiditis characterized by circulating thyroglobulin antibodies and infiltration of the thyroid gland predominantly with mononuclear cells. This experimental model of autoimmune disease is particularly interesting because the cellular events of the immune response are better established in the mouse and genetic influence on

the disease can be readily studied in this species. Indeed, murine thyroiditis was the first example of an experimental autoimmune disease influenced by *H-2*, the major histocompatibility complex of the mouse. Strains of *H-2*^{k} are high-responders and *H-2*^{d} low-responders to thyroglobulin with respect to the titer of thyroglobulin antibody and particularly to the extent of thyroid infiltration[2].

Previous experiments showed that the composition of the antigenic emulsion used for immunization is of utmost importance for the consistent induction of experimental autoimmune thyroiditis in mice[3,4]. Alterations in the composition of the Freund's complete adjuvant and the amount of thyroglobulin used as antigen resulted in large variations in the incidence and severity of the disease (as assessed by the morphology of the thyroid gland[3,4]) as well as in the titer of thyroglobulin antibodies[4]. More recently, lipopolysaccharide was used instead of Freund's complete adjuvant to induce autoimmune thyroiditis[5]. However, mice immunized with homologous thyroglobulin without any adjuvants consistently failed to develop thyroglobulin antibodies or thyroid infiltrates[3,5-7].

The immune mechanisms that lead to the thyroid damage in murine autoimmune thyroiditis are not completely known, however, the role of circulating thyroglobulin antibodies in eliciting thyroid lesions was demonstrated with passive transfer of serum[8]. It was considered that in mice the T lymphocytes are unresponsive to thyroglobulin, i.e., tolerant[9], while thyroglobulin-reactive B lymphocytes, although present, cannot produce thyroglobulin antibodies without the help of T lymphocytes. This help could be obviated by Freund's adjuvant[10,11]. If this were true, then other mechanisms should also be able to trigger B lymphocytes. Therefore, an attempt was made to induce thyroid antibodies and thyroid infiltrates in mice immunized with homologous thyroid extract without any adjuvant, but with the help of an allogeneic effect.

Materials and methods. Female mice of strains AKR/J (*H-2*^{k}), DBA/2J (*H-2*^{d}) as well as their F_1 hybrids were used in all the experiments. The F_1 hybrids are high-responders since the immune responsiveness to thyroglobulin is a genetically dominant trait[2]. The mice were obtained from the Jackson Laboratories, Bar Harbor, ME, and were kept under observation for a week before beginning the experiments. Female F_1 mice were injected i.p., on 3 consecutive days, with 0.5 mg of thyroid extract (in a volume of 0.5 ml) prepared from outbred CF-1 mice as previously described[3]. 2 weeks later, 25×10^6 viable spleen cells from the high-responder female AKR/J parent were injected i.v. into each mouse. Control animals received the same number of spleen cells from syngeneic F_1 mice. 2 days after the cell transfer, all animals were boostered with 0.5 mg thyroid extract, i.e., all animals received a total of 4 injections of aqueous thyroid antigen. Some mice were killed 2 weeks and others 4 weeks after the booster (i.e., 4 and 7 weeks, respectively, after the first injection of antigen). The titer of thyroglobulin antibodies was determined by passive hemagglutination of chromium chloride-treated human red cells coated with purified mouse thyroglobulin as previously described[3,6]. This technique has been routinely used in our laboratory for many years and its specificity was demonstrated by hemagglutination inhibition experiments. The thyroid infiltrations was assessed by microscopic examination of 300 transversal thyroid sections for each animal.

In another experiment, mice were X-irradiated with 250 r, 1 day before the transfer of spleen cells, to enhance the allogeneic effect. In this experiment, a larger number of spleen cells, 100×10^6, were injected i.v. into each animal. The control group received F_1 spleen cells and the allogeneic effect group was injected with parental cells as in the experiment just described.

Results. The control group of mice, which received F_1 spleen cells, did not show thyroglobulin antibodies or thyroid lesions. However, 9 of 13 mice in which an allogeneic effect was probably induced by the parental cell injection showed thyroglobulin antibodies at 2 different times after immunization. Only 1 animal from this group revealed thyroid lesions (table 1).

All animals which were X-irradiated before the injection of parental spleen cells showed small but definite thyroid infiltrates although no thyroglobulin antibodies. In contrast, irradiated control animals which received F_1 spleen cells did not have thyroglobulin antibodies or thyroid infiltrates (table 2).

Discussion. The experiments just described show that thyroglobulin antibodies and thyroid infiltrates can be induced in mice by immunization with homologous mouse thyroid extract (which contains mainly thyroglobulin)[4], without the use of any adjuvant but with the help of an allogeneic effect. The allogeneic effect, initially described in guinea-pigs[12,13], was extensively studied in mice[14-17], and more recently an allogeneic effect factor was isolated[18]. This phenomenon reflects the interaction of allogeneic T lymphocytes with the host T lymphocytes[15]. It appears that the allogeneic effect can trigger thyroglobulin-reactive B cells to produce autoantibodies. An allogeneic effect on cell-mediated immune response was also shown[19] and the existence of thyroglobulin-reactive T lymphocytes was recently postulated[20].

The experimental conditions for producing a helper alloge-

Table 1. Allogeneic effect on induction of thyroglobulin antibodies and thyroid infiltrates in mice*

Animal group		Antibody titer** (positive/total)	Thyroid pathology (positive/total)
Allogeneic effect	(a)	5.0±0.4 (5/6)	1/6
	(b)	2.7±0.4 (4/7)	0/7
Control	(a)	0 (0/6)	0/7
	(b)	0 (0/7)	0/7

* (DBA/2×AKR) F_1 mice were injected i.p. on days 0, 1 and 2 with homologous thyroid extract (0.5 mg/mouse). On day 16, 25×10^6 spleen cells were transferred from AKR mice (allogeneic effect group) or F_1 mice (control group); on day 18, 0.5 mg of antigen was injected i.p. The animals were killed on day 32 (a) or 46 (b). ** Log_2 of thyroglobulin antibodies at the end of the experiments; mean ± SE.

Table 2. Allogeneic effect on induction of murine autoimmune thyroiditis*

Animal group	Antibody titer** (positive/total)	Thyroid pathology positive/total
Allogeneic effect	0 (0/6)	6/6
Control	0 (0/12)	0/12

* (AKR×DBA/2) F_1 mice were injected i.p. on days 0, 1 and 2 with homologous thyroid extract (0.5 mg/mouse); on day 13 they were X-irradiated with 250 r; on day 14 they were injected i.v. with 100×10^6 viable spleen cells from AKR mice (allogeneic effect group) or from F_1 mice (control group). All mice were injected i.p. with 0.5 mg thyroid extract on day 16 and were killed on day 35. ** Log_2 of thyroglobulin antibody titer at the end of experiment; mean ± SE.

neic effect for the induction of antibody are critical since an exagerated or prolonged allogeneic stimulation results in suppression of antibody formation[14]. Since low-dose irradiation increases the allogeneic effect[15], this could explain the absence of thyroglobulin antibodies in X-irradiated mice. Furthermore, low-dose irradiation inhibits suppressor activity[21] and separate suppressor mechanisms for the thyroid lesions and thyroglobulin antibody were described during the induction of autoimmune thyroiditis[22]. The allogeneic effect obtained after irradiation could induce high-affinity thyroglobulin antibodies which are readily fixed by the thyroid and not found in the circulation. They could trigger the induction of thyroid infiltrates. Such a mechanism cannot be firmly proven since the half-life of mouse IgG is only 1.9 days and fluorescent studies on thyroid tissue are not helpful in detecting thyroglobulin antibodies in mice. In any event, the low-dose irradiation per se did not seem to increase the susceptibility of the thyroid to lymphocytic infiltration[7] since control irradiated mice did not show any thyroid damage as late as 22 days after irradiation.

These experiments seem to support the view that normal mice have thyroglobulin-reactive B lymphocytes[9-11]. A graft-versus-host reaction as can develop in humans after bone marrow transplants might stimulate an autoimmune reaction by acting as adjuvant for various autoantigens.

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Effects of epinephrine on plasma fibrinogen levels in rats submitted to tissue injury

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Summary. Tissue injury (laparotomy) produces an increase in plasma fibrinogen. This increase is inhibited by the removal of the adrenal medulla, but injection of epinephrine in laparotomized-medullectomized rats returns fibrinogen levels to values similar to those observed in only laparotomized rats. Epinephrine administration to laparotomized rats increases the fibrinogen compared with the group of laparotomized rats without treatment, but epinephrine by itself does not modify plasma fibrinogen levels in uninjured rats. Epinephrine is apparently responsible for the increase of plasma fibrinogen in rats subjected to tissue injury, probably through beta adrenergic stimulation.

In previous work it has been found that tissue injury increases plasma fibrinogen levels in rats[1-4]. ACTH increases the fibrinogen due to an extra-adrenal mechanism[5] and to an increase of hepatic fibrinogen synthesis[6,7]. On the contrary, adrenalectomy decreases plasma fibrinogen level[8], and corticosterone, which is main glucocorticoid hormone in rats, does not have any influence on it[9]. In the other hand, tissue injury is a noxious stimulus which activates both the sympatoadrenal medullary system and epinephrine secretion; and as the presence of adrenal epinephrine is necessary to produce an increase of alpha globulin in rats submitted to stress[10-13], we have studied, on the basis of these considerations, the role that epinephrine would have on plasma fibrinogen levels in rats submitted to tissue injury.

Material and methods. 133 rats of both sexes, Suquía Strain, weighing 140-220 g were used. They were fed with balanced food containing sufficient protein (20%). The animals were divided into 2 groups: Group I, rats with tissue injury (laparotomy); and group II, rats injected with adrenergic receptor blockers. Each of them included several subgroups. Group I: a) Laparotomized rats without other treatment (L); b) with extirpation of adrenal medulla (LMx); c) with extirpation of adrenal medulla and injected with epinephrine (LMxEp); d) laparotomized rats injected with epinephrine (LEp); e) adrenalectomized rats (LAx); f) normal intact rats: 1. without treatment (control); 2. injected with saline; 3. only with ether anesthesia; 4. injected only with epinephrine. Group II: a) Rats with laparotomy and injection of propranolol (LPr); b) with laparotomy and injection of phenoxybenzamine; c) laparotomized + propranolol + epinephrine (LPrEp); d) laparotomized + phenoxybenzamine + epinephrine (LPhEp); e) normal rats injected with propranolol (Pr) or with phenoxybenzamine.

Tissue injury was made by posterior laparotomy. It included careful manipulation of kidney and adrenal glands. Laparotomy was made with the rats in the prone