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BASOPHILIC LEUCOCYTES AND HASHIMOTO'S THYROIDITIS¹

*Skin Reaction upon Intradermal Injection of Thyroid Extract
Studied by Skin Window Technique*

BY

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Clinical and experimental observations indicate that auto-immune mechanisms may be of importance for the development of Hashimoto's thyroiditis. Circulating antibodies against at least one thyroid constituent are invariably found in patients with this disease, and the disease can be produced in experimental animals by immunization with autologous thyroid extract and Freund's adjuvant.

Even if an auto-immune aetiology is accepted it remains unestablished whether circulating thyroid antibodies or cellular hypersensitivity are of pathogenetic importance. A circulating factor which is specifically toxic to cultured thyroid epithelial cells can be demonstrated in patients with Hashimoto's disease (11, 9) and this cytotoxic factor seems to be identical with one of the serologically demonstrable thyroid antibodies, the complement fixing antibody (10, 5, 7). This observation suggests that the complement fixing antibody is of pathogenetic importance. However, the effect is seen only in cells damaged by trypsin (10, 7) and attempts of trans-

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ferring thyroiditis to normal animals by means of serum containing thyroid antibodies have been unsuccessful (14).

The constant presence of lymphocytes and plasma cells at the site of the lesion suggests that cellular hypersensitivity plays a role in the development of chronic thyroiditis. Transfer of the disease to healthy animals by means of lymphoid cells from sensitized donors has been reported (4). Positive skin tests have been found following intradermal injection of thyroid extract into patients with thyroid disease and circulating thyroid antibodies (3). However, the significance of the finding remains unclear since the skin reaction was interpreted as an Arthus phenomenon (3).

The aim of this paper is to decide, by means of the skin window technique, whether the skin reaction is a tuberculoid type reaction or an Arthus phenomenon. The skin window technique has not previously been used for the study of a disease of possible autoimmune etiology.

MATERIAL AND METHODS

The skin window technique was applied to seven patients with Hashimoto's thyroiditis. The diagnosis had been verified in each case by means of a thyroid biopsy. All patients were women aged 35 to 61 years. 4 patients were euthyroid, 3 had developed myxoedema and received thyroid medication. The possibility exists that the injection of thyroid extract into euthyroid patients with chronic thyroiditis might cause a flare-up of the disease, thus making the patients myxoedematous. Consequently, their thyroid function was followed closely. Six control patients without evidence of thyroid disease, 4 women and 2 men, were also included in the experiment.

The demonstration of microsomal circulating antibodies was done by an immune fluorescence technique as described by *Holborrow, Brown, Roitt & Doniach* (8). Positive reactions were titrated by means of a complement fixation reaction described by *Roitt & Doniach* (14) using standardized extracts of toxic goitres as antigen. Thyroglobulin antibody was demonstrated by thyroglobulin—sensitized sheep cells obtained from Bourroughs Wellcome & Co. CA-2 antibody was demonstrated by an immune fluorescence technique as described by *Balfour, Doniach, Roitt & Couchman* (2).

Thyroid extracts for the skin reaction were made using the method of *Goudie, Anderson, Gray, Clark, Murray & McNicol* (6). The extracts were Seitz filtered and the sterility checked by bacterial culture.

The skin window examination was performed using the method of *Rebuck*,

Table 1
Circulating Thyroid Antibodies and Maximum Percentages of Basophilic and Eosinophilic Leucocytes Following Injection of Thyroid Extract and Saline. 7 Patients with Chronic Thyroiditis (1-7) and 6 Control Persons (I-VI).

Chronic thyroiditis number of patients	Cytoplasmic fluorescence	Complement fixing titre	CA-2 antibody	Thyroglobulin antibody	Thyroid extract		Saline	
					Maximum percentage basophils	Maximum percentage eosinophils	Maximum percentage basophils	Maximum percentage eosinophils
1	+	512		2,500,000	8.5	4.5	0.5	0.5
2	+	512		250	3.0	3.5	1.0	0
3	+	128		2,500	3.0	1.0	1.0	0
4	+	64		250	3.0	2.0	1.0	0
5	+	—		2,500,000	2.0	4.0	1.0	0.5
6	+	256		25,000	4.0	4.5	0	0
7	+	64		250	3.5	4.0	0.5	0.5
Number of control persons								
I	—		—	—	0.5	0	0.5	0
II	—		—	—	0.5	0	0.5	0
III	—		—	—	1.0	0.5	0.5	0
IV	—		—	—	1.0	0.5	0	0
V	—		—	—	0.5	0	0.5	0
VI	—		—	—	0	0	0.5	0

Petz, Riddle, Priest & LoGrip (12). 0.1-0.2 ml thyroid extract was injected into a denuded area of the skin of the volar side of one forearm. Symmetrically on the other arm the same volume of saline was injected as control. The areas were covered by sterile coverslips. The coverslips were changed every three hours throughout the following 48 hours. The cells of the exudate adhering to the coverslips were stained with May-Grünwald-Giemsa, and differential counts of the cells of each coverslip were performed.

Furthermore 0.1 to 0.2 ml of the same extract was injected intradermally at a distance of at least 7 cm from the corresponding skin window. The gross reactions were read after 48 hours.

RESULTS

In the skin windows of all the seven patients with chronic thyroiditis, basophilic leucocytes appeared following injection of thyroid extract. The basophilic leucocytes were particularly numerous on the second day of the experiments when they amounted to 1.5 to 8.5 per cent of the cells of the exudate.

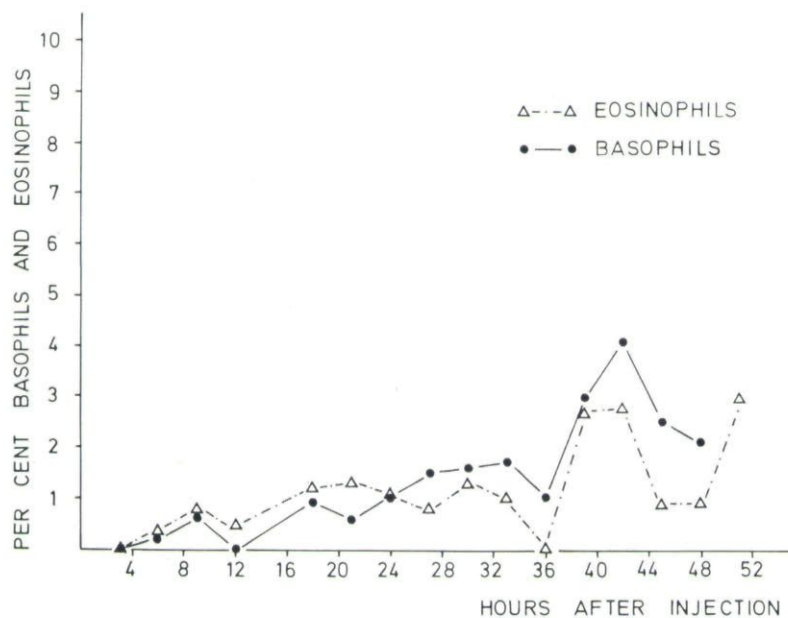


Fig. 1.

Average percentages of basophilic and eosinophilic leucocytes in skin windows following injection of thyroid extract into seven patients with chronic thyroiditis.

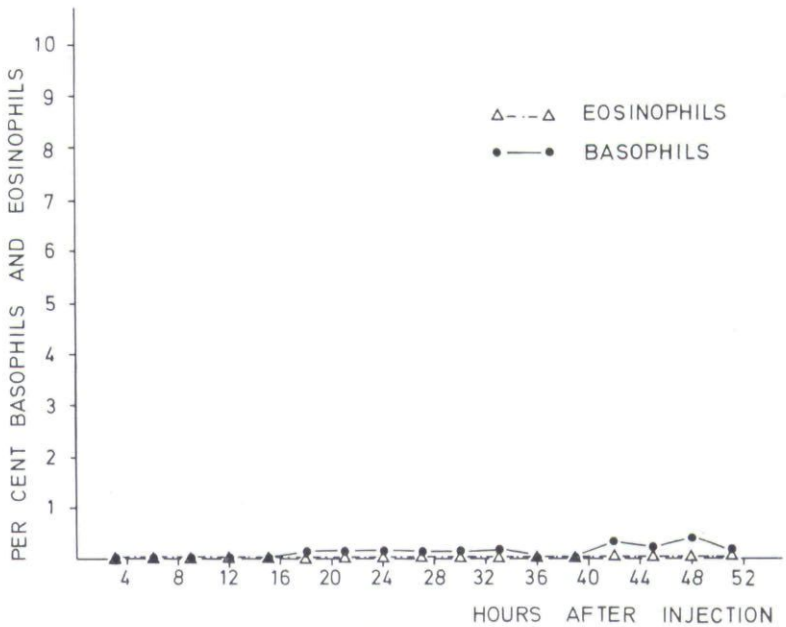


Fig. 2.

Average percentages of basophilic and eosinophilic leucocytes in skin windows following injection of saline into seven patients with chronic thyroiditis.

All of the seven control experiments on the same patients revealed no or very few (max. of 0 to 1.5 per cent) basophilic leucocytes following injection of saline. A number of eosinophilic leucocytes appeared in the exudate at the same time as the basophils following injection of thyroid extract, whereas neutrophils and macrophages appeared in much the same number and sequence after injection of thyroid extract and saline.

Table 1 shows the findings of circulating antibodies and the maximum percentages of basophils and eosinophils in the skin windows after injection of thyroid extracts and saline into the seven patients with Hashimoto's thyroiditis compared with six control subjects. Fig. 1 shows the average number of basophilic and eosinophilic leucocytes in the skin windows following injection of thyroid extract into the seven patients with

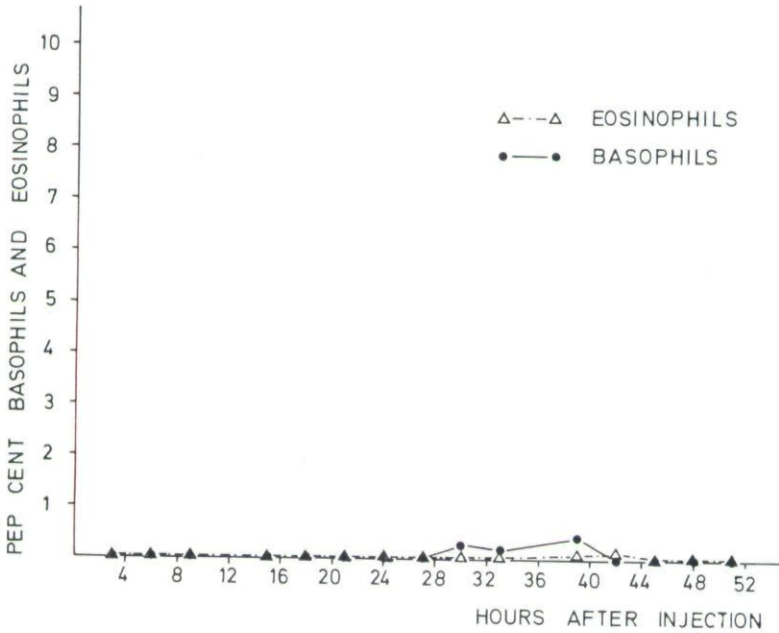


Fig. 3.

Average percentages of basophilic and eosinophilic leucocytes following injection of thyroid extract into six control persons.

chronic thyroiditis. A maximum of 4.2 and 2.8 per cent, respectively, was reached on the second day of the experiments. Fig. 2 shows that hardly any basophils or eosinophils appeared in the skin windows of the same patients following injection of saline. Figs. 3 and 4 show that no basophils or eosinophils appeared in the skin windows of the control persons receiving injections of thyroid extract and saline.

The gross reactions were difficult to read. Two of the patients with chronic thyroiditis had negative reactions, five patients revealed erythema, the extent of which varied from 6 to 14 mm. Infiltration of the skin was seen in none of the cases.

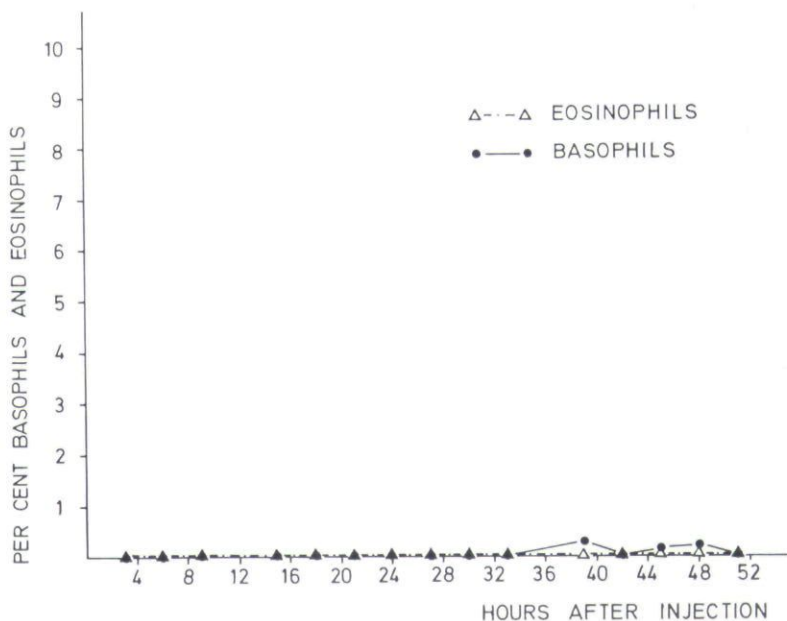


Fig. 4.

Average percentages of basophilic and eosinophilic leucocytes in skin windows following injection of saline into six control persons.

DISCUSSION

Previous work on the skin window technique has shown that basophilic leucocytes were characteristic of delayed types of hypersensitivity reactions of the tuberculin types (1, 17) experimental contact dermatitis (15), and the normal-lymphocyte transfer test (NLT-test) (16). Basophilic leucocytes are not seen in non-specific inflammation (13). In the present study the skin windows revealed basophilic leucocytes following intradermal injection of thyroid extract into patients with Hashimoto's thyroiditis and circulating thyroid antibodies, whereas no basophils were found in the skin windows following injection of thyroid extract into normal control persons.

This observation indicates that the positive skin reaction seen in patients with thyroid disease following intradermal injection of thyroid extract as described by *Buchanan, Ander-*

son, Goudie & Gray (3) is a delayed type hypersensitivity reaction and not an Arthus phenomenon. Consequently, cellular antibodies seem to be of importance for the development of chronic thyroiditis, and basophilic leucocytes may play a role in auto-immune reactions.

SUMMARY

The cellular response to thyroid antigens in patients with a disease of possible auto-immune etiology was studied by the skin window technique. Seven patients with Hashimoto's thyroiditis and circulating thyroid antibodies received intradermal injections of thyroid extract. The exudate contained basophilic leucocytes, the number of which reached a maximum on the second day of the experiment. No basophils were seen in the exudate after injection of saline or following injection of thyroid extract into six control persons without thyroid disease and without circulating thyroid antibodies. A number of eosinophilic leucocytes appeared in the exudate at the same time as the basophilic cells. Since basophilic leucocytes have previously been found in delayed type hypersensitivity reactions but not in non-specific inflammation in skin windows, this observation suggests that the skin reaction is of a delayed type. Consequently, cellular antibodies against thyroid antigens may occur in Hashimoto's thyroiditis, and basophilic leucocytes may be of importance in auto-immune reactions.

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