Clin. exp. Immunol. (1973) **14,** 153–158.

LYMPHOCYTE-DEPENDENT ANTIBODY-MEDIATED CYTOTOXICITY IN HASHIMOTO THYROIDITIS

ELIZABETH A. CALDER, W. J. PENHALE, DENA McLEMAN, E. W. BARNES AND W. J. IRVINE

Department of Endocrinology, Royal Infirmary, Edinburgh; and Department of Therapeutics, University, Edinburgh

(*Received* 26 *January* 1973)

SUMMARY

In the presence of normal human lymphocytes, decomplemented sera from twenty-nine out of thirty-nine patients with Hashimoto thyroiditis caused significant lysis of thyroglobulin-coated chicken red blood cells, as estimated by the release of ^{51}Cr; the mean % specific ^{51}Cr release being $14{\cdot}1 \pm 1{\cdot}9$ (SEM). Serum from twenty-one control subjects studied concurrently caused no significant lysis of thyroglobulin-coated chicken red blood cells; the mean % specific ^{51}Cr release being $-1{\cdot}6 \pm 0{\cdot}7$ (SEM).

The degree of cytotoxicity correlated with the titre of thyroglobulin antibodies in the serum, determined by tanned red cell haemagglutination. The active component in the Hashimoto serum was localized in the 19S fraction, was unaffected by pre-absorption with anti-human IgM serum, but was neutralized by pre-absorption with anti-human IgG serum. These findings suggest that the cytotoxic activity of serum from patients with Hashimoto thyroiditis is due to the presence of thyroglobulin antibody of the IgG class in the form of complexes, either alone or with antigen.

It is postulated that non-specific lymphocytes may play an important role in the pathogenesis of Hashimoto thyroiditis, being activated by the presence in the gland of thyroglobulin antibody, either alone or in the form of complexes attached to thyroid cells.

INTRODUCTION

In vitro experiments have suggested that humoral and cellular components may play a role in the pathogenesis of Hashimoto thyroiditis. For example, it has long been known that in the presence of complement sera from patients with Hashimoto thyroiditis can cause the destruction of thyroid cells in culture (Pulvertaft *et al.*, 1961; Irvine, 1960).

Also several workers have shown that patients with Hashimoto thyroiditis possess lymphocytes which are specifically sensitized to components of the thyroid gland and are

Correspondence: Dr W. J. Irvine, Department of Endocrinology, Royal Infirmary, Edinburgh EH3 9YW.

capable of causing cell damage (Podleski, 1972; Calder *et al.*, 1973). As yet, however, no convincing evidence exists that either humoral or cellular mechanisms alone play a primary pathogenic role *in vivo*.

The concept of lymphocyte-dependent antibody-mediated cytotoxicity was first explored by Perlman & Holm (1969), Granger & Kolb (1968) and MacLennan & Loewi (1968) who demonstrated that in a model system non-specific lymphocytes caused the lysis of target cells, such as chicken red cells or Chang liver cells coated with target cell specific antibody.

Experiments of Ringertz *et al.* (1971) have shown that sera from guinea-pigs with experimental allergic thyroiditis rendered lymphocytes from normal animals cytotoxic to thyroglobulin-coated target cells.

The possibility that a similar mechanism may play a role in the pathogenesis of Hashimoto thyroiditis has not been explored, although preliminary experiments of Fakhri & Hobbs (1972) have shown that when normal rat lymphocytes are added to antibody-coated cryostat sections of human thyroid gland, the lymphocytes became localized in the follicular lining of the thyroid tissue and caused thyroid cell death within 20 hr.

The present paper describes the use of chicken red blood cells labelled with ^{51}Cr and coated with thyroglobulin to detect lymphocyte-dependent antibody-mediated cytotoxicity in Hashimoto thyroiditis.

MATERIALS AND METHODS

Chicken red blood cells (Ch. RBC)

Ch. RBC were coated with thyroglobulin and labelled with ^{51}Cr (Calder *et al.*, 1973). The prepared cells were divided into three aliquots each containing approximately 2×10^6 cells in a volume of 0·1 ml. Each aliquot was incubated at 37°C for 30 min; one with Hashimoto serum, the second with control serum and the third with buffer, each in a volume of 0·1 ml. The cells were then washed three times in tissue culture medium (Eagles MEM, Wellcome Reagents Ltd), counted and left pelletilized at room temperature until required (up to 2–3 hr).

Lymphocytes

Lymphocytes were separated from whole blood by density centrifugation on a Ficoll–Triosil gradient (Calder *et al.*, 1973), washed in tissue culture medium with 10% foetal calf serum, counted, and adjusted to 5×10^6 cells/ml.

Culture conditions

Cultures were set up in Sterilin tissue culture tubes (115 × 13 mm) in triplicate, each containing 10^5 target cells and $2{\cdot}5 \times 10^6$ lymphocytes in a total volume of 1·5 ml tissue culture medium supplemented with 10% foetal calf serum. One group contained buffer-treated target cells, the second, target cells pre-incubated with Hashimoto serum and the third, target cells pre-incubated with control serum. In addition, control cultures were set up in duplicate containing 10^5 target cells and $2{\cdot}5 \times 10^6$ washed, unlabelled Ch. RBC in place of the lymphocytes. All cultures were incubated at 37°C for 18 hr in air—5% CO_2.

Assay procedure and calculation of cytotoxicity

After incubation, the cultures were centrifuged at 200 *g* for 10 min and 1·0 ml supernatant

removed. ^{51}Cr release into the supernatant and the residual ^{51}Cr left in the culture pellet was counted in a well-type automatic gamma counter (Wallac). The % isotope release was calculated as follows:

$$\frac{\text{Mean supernatant count} \times 1{\cdot}5}{\text{Mean supernatant count} + \text{mean pellet count}} \times 100.$$

For both lymphocyte cultures and lymphocyte-free control cultures, the specific % ^{51}Cr release due to the presence on the target cells of absorbed serum factors was estimated by subtracting the % ^{51}Cr release obtained with buffer-treated target cells.

Serum fractionation procedure

Whole serum was separated into 19S, 7S and albumin components by column chromatography using Sephadex G-200. The effect of serum fractions on antigen-coated chicken red blood cells was estimated in an identical manner to whole serum. Serum fractions which had been pre-absorbed with anti-human γ or anti-human μ chain antisera (goat anti-human IgG serum and goat anti-human IgM serum; Wellcome Reagents Ltd, Cat. No. MR 54 and MR 55 respectively) were also tested. Absorption was effected by incubating equal volumes of serum and 1:2 diluted antiserum for 2–3 hr at 37°C.

Serology

All sera were tested and titred for thyroid and mitochondrial antibodies by indirect immunofluorescence using unfixed sections of human thyroid and rat kidney and by tanned red cell haemagglutination and complement fixation using purified thyroglobulin and thyroid extract respectively.

PATIENTS

Serum samples from thirty-nine patients with proven Hashimoto thyroiditis were included in this study. Histological confirmation of the diagnosis was available in twenty of the thirty-nine patients. In the remaining nineteen patients, diagnosis was made on the basis of a goitre and characteristic serology (either positive immunofluorescence tests for thyroid microsomal antibody with a complement fixation titre of $\geqslant 1/32$, or a tanned red cell haemagglutination titre of $\geqslant 1/2{,}500$ or both).

Serum samples from thirteen of the thirty-nine patients were taken at the time of diagnosis. Twenty-six patients were on thyroxine therapy at the time the serum sample was taken, the period of treatment ranging from 1 to 11 years.

Serum samples from twenty-one control subjects, consisting of medical and laboratory personnel, were studied concurrently. All were negative for thyroid antibodies.

Lymphocytes were obtained from a pool of thirty control subjects, again consisting of medical and laboratory personnel.

RESULTS

The effect of normal human lymphocytes on thyroglobulin-coated Ch. RBC pre-incubated with either control serum or Hashimoto serum is shown in Fig. 1. The sera from the thirty-nine patients with Hashimoto thyroiditis gave a mean % specific ^{51}Cr release of $14{\cdot}1 \pm 1{\cdot}9$

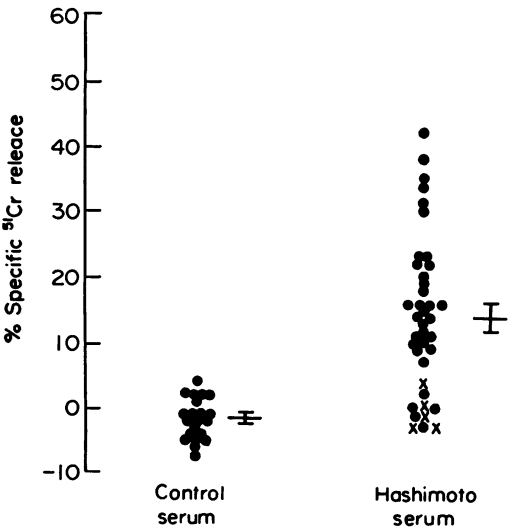

FIG. 1. The lymphocyte-dependent cytotoxicity of serum from control subjects and patients with Hashimoto thyroiditis.
× = negative tanned cell haemagglutination titre for antibody to thyroglobulin in the Hashimoto patient's serum.

(SEM). The sera from the twenty-one control subjects studied concurrently gave a mean % specific ^{51}Cr release of $-1{\cdot}6 \pm 0{\cdot}7$ (SEM). The observed lymphocyte-dependent cytotoxic activity of Hashimoto serum was highly significant ($P \geqslant 0{\cdot}001$). In the absence of lymphocytes neither control nor Hashimoto serum caused significant lysis of thyroglobulin coated Ch. RBC.

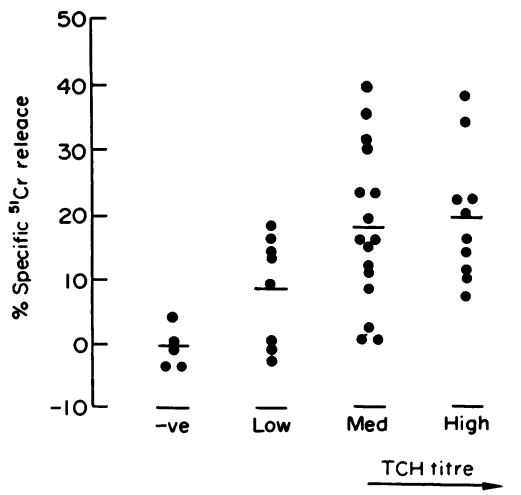

FIG. 2. The relationship between tanned cell haemagglutination (TCH) titre for thyroglobulin antibody and cytotoxicity. Low >25 <2,500; medium ⩾2,500 <250,000; high ⩾250,000.

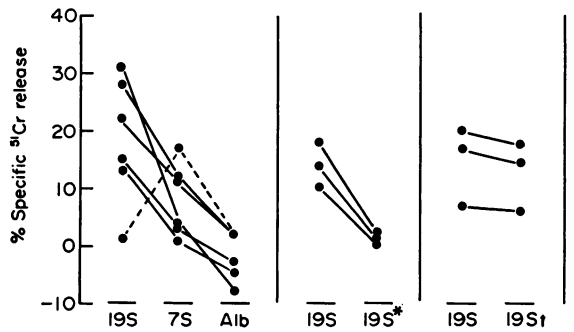

FIG. 3. The lymphocyte-dependent cytotoxicity of serum components in Hashimoto thyroiditis.
* Absorbed with human anti-IgG. † Absorbed with human anti-IgM.

Fig. 2 shows the correlation between serum cytotoxicity and tanned red cell haemagglutinating titre. The regression coefficient was significant at $P \leqslant 0{\cdot}01$.

In an attempt to characterize the cytotoxic activity of whole serum, six randomly selected Hashimoto serum samples were separated into 19S, 7S and albumin components by Sephadex G-200 chromatography. Their effect on thyroglobulin coated chicken red blood cells is shown in Fig. 3. In five of the six serum samples, the lymphocyte-dependent cytotoxic activity was found in the 19S fraction, only slight activity being detectable in the 7S fraction, and no activity being present in the albumin fraction. With one serum sample activity was greatest in the 7S fraction, the 19S and albumin components being inactive. In the absence of lymphocytes none of the three serum components caused lysis of target cells.

It seemed therefore that the cytotoxicity of Hashimoto serum was due to thyroglobulin antibody of the IgM class. Contrary to expectation, however, the cytotoxic activity of the 19S fraction was unaffected by pre-absorption with anti-human IgM serum but was neutralized by pre-absorption with anti-human IgG serum.

DISCUSSION

The results of the present *in vitro* experiments show that sera from patients with Hashimoto thyroiditis contain a factor which, in conjunction with non-immune lymphocytes, causes the destruction of thyroglobulin-coated Ch. RBC.

Although our studies on the characterization of the lymphocyte-dependent cytotoxic activity of Hashimoto serum are still in the preliminary stages, results suggest that the activity is due to the presence of thyroglobulin antibody in the IgG class. The finding of the IgG activity in the 19S fraction of whole serum could be the result of complex formation either with itself or with antigen.

In the model system thyroglobulin antibody, either alone or in the form of complexes, combines with the thyroglobulin on the red cell surface with subsequent activation of the Fc portion of the antibody. It is postulated that non-specific lymphocytes then combine with the activated Fc portion resulting in the death of the target cell (Fakhri & Hobbs, 1972).

In vivo, a similar process may be operating insofar that thyroglobulin is freely available in the colloid and presumably on the surface of the thyroid cell and is known to escape from the thyroid into the surrounding tissues. It is therefore likely that some may become adherent to the thyroid cell membrane and be accessible to thyroglobulin antibody either

synthesized within the thyroid itself or transported by the blood stream. What we have studied in the model system could then occur within the thyroid.

One might postulate that in Hashimoto thyroiditis, and presumably in certain other autoimmune diseases, the initial derangement is a genetically determined defect in immunological tolerance whereby specifically sensitized T and B lymphocytes are produced in relation to thyroid antigens. The T lymphocytes may cause damage to the thyroid following infiltration and release of lymphokines (Dumonde *et al.*, 1969) and the B lymphocytes would lead to the synthesis of thyroglobulin and other antibodies. This in turn would produce conditions whereby a much larger population of non-specific lymphocytes could cause tissue damage by the mechanisms described in this paper.

Alternatively, thyroglobulin antibody complexed with thyroglobulin may sensitize non-specific lymphocytes in the circulation thus giving them specificity to destroy thyroid cells coated with either thyroglobulin or thyroglobulin antibody. This possibility is currently being investigated.

ACKNOWLEDGMENT

This work was supported by the Medical Research Council.

REFERENCES

CALDER, E.A., PENHALE, W.J., BARNES, E.W. & IRVINE, W.J. (1973) Cytotoxic lymphocytes in Hashimoto thyroiditis. An *in vitro* assay system using ^{51}Cr-labelled chicken red blood cells coated with thyroglobulin. *Clin. exp. Immunol.* **14**, 19.

DUMONDE, D.C., WOLSTENCROFT, R.A., PANAYI, G.S., MATTHEW, M., MORLEY, J. & HOWSON, W.T. (1969) 'Lymphokines.' Non-antibody mediators of cellular immunity generated by lymphocyte activation. *Nature (Lond.)*, **224**, 38.

FAKHRI, O. & HOBBS, J.R. (1972) Detection of antibodies which can co-operate with lymphocytes. *Lancet*, **ii**, 403.

GRANGER, G.A. & KOLB, W.P. (1968) Lymphocyte *in vitro* cytotoxicity mechanisms of immune and non-immune small lymphocyte mediated target cell destruction. *J. Immunol.* **101**, 111.

IRVINE, W.J. (1960) The cytotoxic factor in thyroid disease. *Scot. med. J.* **5**, 511.

MACLENNAN, I.C.M. & LOEWI, C. (1968) The effect of specific antibody to target cells on their specific and non-specific interaction with lymphocytes. *Nature (Lond.)*, **219**, 1069.

PERLMAN, P. & HOLM, G. (1969) Cytotoxic effects of lymphoid cells *in vitro*. *Advanc. Immunol.* **11**, 117.

PODLESKI, N.K. (1972) Cytotoxic lymphocytes in Hashimoto thyroiditis. *Clin. exp. Immunol.* **11**, 543.

PULVERTAFT, R.V., DONIACH, D., ROITT, I.M. & HUDSON, R.V. (1961) The cytotoxic factor in Hashimoto's disease and the incidence in the other thyroid diseases. *Brit. J. exp. Path.* **42**, 496.

RINGERTZ, B., WASSERMAN, J., PACKALEN, T.L. & PERLMAN, P. (1971) Cellular and humoral responses in experimental autoimmune thyroiditis. *Int. Arch. Allergy*, **40**, 917.