Clin. exp. Immunol. (1973) **15**, 467–470.

SHORT COMMUNICATION

LYMPHOCYTE CYTOTOXICITY INDUCED BY PRE-INCUBATION WITH SERUM FROM PATIENTS WITH HASHIMOTO THYROIDITIS

ELIZABETH A. CALDER, DENA McLEMAN AND W. J. IRVINE

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

(*Received* 16 *July* 1973)

SUMMARY

Lymphocytes from healthy donors were incubated with serum samples from nine patients with Hashimoto thyroiditis and subsequently shown to be cytotoxic to chicken red blood cells (Ch. RBC) coated with thyroglobulin. Target cell death was estimated using a standard ^{51}Cr release assay system.

Lymphocytes pre-incubated with Hashimoto serum caused a mean % ^{51}Cr release of $13{\cdot}11 \pm 2{\cdot}83$ (SEM) from thyroglobulin-coated Ch. RBC and a mean % ^{51}Cr release of $1{\cdot}22 \pm 0{\cdot}65$ from uncoated Ch. RBC.

Untreated lymphocytes caused no significant isotope release from either uncoated or thyroglobulin coated target cells.

INTRODUCTION

It is now well established that the addition of normal lymphocytes to target cells coated with anti-target cell antibody results in the death of the target cell (Perlmann & Holm, 1969; Granger & Kolb, 1968; MacLennan & Loewi, 1968). The possibility that cell-mediated antibody-dependent cytotoxic mechanisms are important in the pathogenesis of autoimmune thyroid disease has recently been explored by Calder *et al.* (1973a) who showed that normal lymphocytes are cytotoxic to Ch. RBC coated with thyroglobulin and thyroglobulin antibody. Serum from patients with Hashimoto thyroiditis was used as a source of thyroglobulin antibody. Subsequent analysis of the Hashimoto serum showed that the active cytotoxic factor was a thyroglobulin antibody of the IgG class. Its presence in the 19S fraction of whole serum was postulated as being due to complex formation. It was further postulated that such immune complexes could play an important pathogenic role *in vivo* by specifically activating lymphocytes to destroy thyroid cells.

Preliminary experiments of Perlmann, Perlmann & Biberfield (1972) have shown that lymphocytes which have been incubated with antibody-complexed chicken erythrocytes are highly cytotoxic to freshly added Ch. RBC. Lymphocytes incubated with either ery-

Correspondence: Dr W. J. Irvine, Department of Endocrinology, The Royal Infirmary, Edinburgh, Scotland.

throcytes or erythrocyte antibody alone do not show this cytotoxic capacity. On the basis of these results it would appear that lymphocytes are capable of being specifically activated by immune complexes.

The aim of the present investigation was to see whether lymphocytes which had been incubated with serum from patients with Hashimoto thyroiditis were subsequently cytotoxic to thyroglobulin-coated target cells.

MATERIALS AND METHODS

1. *Target cells*

These were chicken red blood cells which were coated with thyroglobulin and labelled with ^{51}Cr (Calder *et al.*, 1973b). For use in control cultures, uncoated Ch. RBC were also labelled with ^{51}Cr.

2. *Hashimoto serum*

Sera was obtained from patients with proven Hashimoto thyroiditis. Serum samples had previously been tested and shown to be active in the cell-mediated antibody-dependent cytotoxicity test (Calder *et al.*, 1973a). Furthermore, the activity of the selected serum samples was known to be localized exclusively in the 19S fraction. Serum was not heat inactivated but had been absorbed twice at 4°C against an equal volume of packed Ch. RBC. Serum tanned cell haemagglutination titres ranged from 25,000 to 2,500,000.

3. *Lymphocytes*

These were separated from whole blood of healthy volunteers by centrifugation on a Ficoll–Triosil gradient (Calder *et al.*, 1973b) washed in tissue culture medium (Eagles, MEM, Wellcome Reagents Ltd), supplemented with 10% heated foetal calf serum (FCS, Wellcome Reagents Ltd) and counted. Lymphocytes were then divided into two aliquots and incubated in tissue culture tubes (Sterilin Ltd) for 2 hr at 37°C. One tube contained 30×10^6 viable lymphocytes in 2 ml tissue culture medium (subsequently referred to as 'untreated'). The second tube contained 30×10^6 viable lymphocytes in 2 ml culture medium supplemented with 20% Hashimoto serum (subsequently referred to as 'pre-incubated'). After incubation, lymphocytes were washed four times in culture medium supplemented with 10% FCS and adjusted to 5×10^6 viable cells/ml.

4. *Assay procedure*

Cultures were set up in triplicate each containing 10^5 target cells and $2{\cdot}5 \times 10^6$ lymphocytes in a total volume of 1·5 ml culture medium supplemented with 10% heat-inactivated FCS.

Both untreated and pre-incubated lymphocytes were tested against thyroglobulin-coated target cells and against uncoated target cells. 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 lymphocytes. In addition the cell-free supernatant from the pre-incubated lymphocyte suspension was cultured with untreated lymphocytes and thyroglobulin coated target cells to ensure that any observed cytotoxicity of the pre-incubated lymphocytes was not due simply to a carry-over of free thyroglobulin antibody.

All cultures were incubated at 37°C for 18 hr in air, 5% CO_2.

5. *Assay procedure and calculation of cytotoxicity*

The mean % isotope release for each set of triplicate cultures was calculated exactly as described previously (Calder *et al.*, 1973b). The net % specific isotope release from both thyroglobulin coated and uncoated target cells due to the presence of absorbed serum factors on the lymphocytes was estimated by subtracting the absolute % ^{51}Cr release obtained with untreated lymphocytes from the absolute % release obtained with pre-incubated lymphocytes.

RESULTS

The effect of incubation of lymphocytes with Hashimoto serum on the release of isotope from both thyroglobulin coated and uncoated Ch. RBC is shown in Fig. 1.

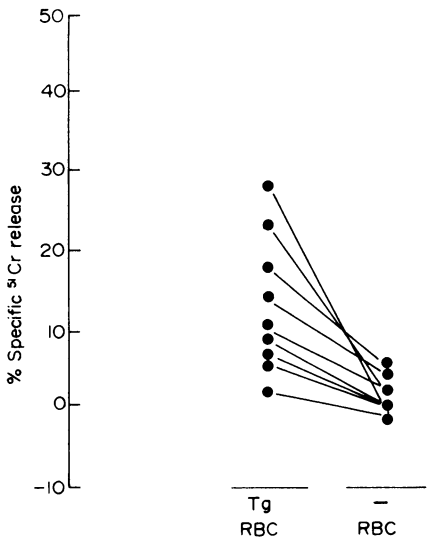

FIG. 1. The effect of lymphocytes pre-incubated with Hashimoto serum on the release of ^{51}Cr from chicken red blood cells coated with thyroglobulin and from uncoated chicken red blood cells.

Incubated lymphocytes caused no significant release of isotope from uncoated target cells. The mean % specific release was $1{\cdot}22 \pm 0{\cdot}65$. In contrast, lymphocytes pre-incubated with Hashimoto serum caused a highly significant release of isotope from thryoglobulin-coated target cells, ranging from +2% to +28%, the mean % specific release being $+13{\cdot}11 \pm 2{\cdot}83$.

The coefficient of variation between replicate cultures is 3–4% and the significance level is 7–8%. Six of the nine serum samples tested were therefore capable of specifically activating lymphocytes to destroy target cells coated with thyroglobulin. Furthermore, addition of a cell-free supernatant from the pre-incubated lymphocytes to untreated lymphocytes did not cause cytotoxicity, showing that the observed activity was not due to a carry-over of free thyroglobulin antibody.

DISCUSSION

The results of this preliminary investigation suggest that human peripheral blood lymphocytes are capable of being specifically activated by components present in Hashimoto serum to destroy thyroglobulin coated target cells.

The identification of the lymphocyte activating serum component is obviously important. That it is a thyroglobulin–thyroglobulin antibody complex is at present somewhat hypothetical. Lymphocytes have low affinity for cytophilic antibody unreacted with antigen (Coombs & Franks, 1969). It is therefore unlikely that lymphocytes gain their cytotoxic potential by mere antibody absorption. As with macrophages, however, certain lymphocytes may have higher affinity for antibody complexed to antigen (Coombs & Franks, 1969). Interaction of these lymphocytes with such complexes may then render them specifically cytotoxic.

This cytotoxic mechanism could prove to be of major significance in the pathogenesis of autoimmune thyroid disease since, *in vivo*, a high proportion of circulating lymphocytes could thus be specifically recruited to destroy thyroglobulin coated cells in the thyroid gland.

Experiments are currently being performed to identify and characterize the lymphocyte activating component present in the serum of patients with Hashimoto thyroiditis.

REFERENCES

CALDER, E.A., PENHALE, W.J., MACLEMAN, D., BARNES, E.W., & IRVINE, W.J. (1973a) Lymphocyte-dependent antibody mediated cytotoxicity in Hashimoto thyroiditis. *Clin. exp. Immunol.* **14,** 153.

CALDER, E.A., PENHALE, W.J., BARNES, E.W. & IRVINE, W.J. (1973b) 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.

COOMBS, R.R.A. & FRANKS, D. (1969) Immunological reactions involving two cell types. *Progr. Allergy,* **13,** 174.

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.

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.

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

PERLMANN, P., PERLMANN, H. & BIBERFIELD, P. (1972) Specifically cytotoxic lymphocytes produced by pre-incubation with antibody complexed target cells. *J. Immunol.* **108,** 558.