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T, B and K cells in autoimmune thyroid disease

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SUMMARY

The K-cell cytotoxic activity of peripheral blood lymphoid cells from 104 patients with autoimmune thyroid disease and from age and sex matched control subjects was measured using chicken erythrocytes as target cells. Patients with Hashimoto thyroiditis, primary hypothyroidism and thyrotoxicosis who were either newly diagnosed and untreated or had received therapy for ≤ 1 year showed a significant increase in K-cell cytotoxic activity. Patients who had received treatment for >1 year and ≤ 5 years showed no such comparable increase in cytotoxic activity.

Within the group of patients with untreated thyrotoxicosis it was found that K-cell cytotoxic activity was related to both goitre size and serum antibody titre. Thus patients with little or no goitre showed a highly significant elevation of cytotoxic activity whereas patients with moderate to large goitres gave values within the normal range. Similarly patients with no detectable serum thyroid autoantibodies showed high K-cell activity while patients with positive antibody titres did not.

It was also shown that neither the absolute number nor the proportion of circulating T and B lymphocytes in patients with autoimmune thyroid disease as assessed by the sheep red cell rosette method and by indirect immunofluorescence was significantly different from that observed in the normal control population.

No correlation was found between peripheral blood K-cell cytotoxic activity and the percentage of circulating null cells, i.e. 100−(percentage T+percentage B) in either patients or control subjects.

INTRODUCTION

K cells belong to neither the mature B- nor T-lymphocyte population and may be defined as a subpopulation of lymphoid cells or monocytes which *in vitro* have the capacity to destroy antibody coated target cells. The characteristics of K cells and their cytotoxic activity have been reviewed (MacLennan, 1972; Perlmann, Perlmann & Müller-Eberhard, 1973; Wisloff, Michaelson & Froland, 1974; Calder *et al.*, 1974).

The precise biological significance of K cell-mediated cytotoxic reactions is still uncertain although the suggestion has been put forward that they are of importance in tumour rejection and in tissue damaging autoallergic disease (Pollack *et al.*, 1972; Lamon *et al.*, 1973; Calder *et al.*, 1973a; Wassermann *et al.*, 1974).

The purpose of this study was to estimate K-cell activity and T- and B-cell numbers in the peripheral blood of patients with autoimmune thyroid disease and of healthy control subjects.

PATIENTS, MATERIALS AND METHODS

(i) *Patients.* Thyroid patients attending the Royal Infirmary of Edinburgh for diagnostic investigations before treatment, or for follow-up within 5 years of diagnosis, were selected according to the following criteria after full investigation (radio-

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iodine uptake, total plasma thyroxine and triiodothyronine, effective thyroxine ratio, and TSH measurements, with or without TRH stimulation as appropriate).

(1) *Thyrotoxicosis.* Twenty-three patients (F 20, M 3) clinically and on investigation unequivocally thyrotoxic; three were taking propranolol, but none had had surgery, radio-iodine or anti-thyroid drugs. Goitre size was arbitrarily graded 0 to +++ by one observer (N.McD.D.). (Age 13–66 years, mean 34·8 years.)

(2) *Treated thyrotoxicosis.* Twenty-seven patients (F 20, M 7) treated with ^{131}I alone for unequivocal thyrotoxicosis at least 6 months before; two had needed a second dose of ^{131}I; none were thyrotoxic at the time of sampling, but one was hypothyroid. (Age 40–64 years, mean 50·2 years.)

(3) *Hashimoto's thyroiditis.* Female goitrous patients diagnosed on thyroid biopsy (five), on serological criteria (TCH> 1:2500 and/or CFT> 1:32) (twenty), or on the concurrence of goitre, hypothyroidism and lower titres of anti-thyroid antibodies (three). Three were hypothyroid; the remainder were euthyroid and, except one, were all taking thyroxine. (Age 40–71 years, mean 44·6 years.)

(4) *Primary hypothyroidism.* Twenty-six patients (F 19, M 7) clinically and on investigation unequivocally hypothyroid, and who had never had a goitre as far as could be established. Eight were untreated and hypothyroid, and the remainder euthyroid and taking thyroxine. (Age 14–82 years, mean 61·8 years.)

Matched control samples were obtained for each patient on the day of sampling from unrelated friends of patients, hospital employees, voluntary workers and thirteen geriatric patients, none of whom had a history of thyroid or other autoimmune disease, and who were within 6 years of the age of the patient. All patients and controls were Caucasian, and none were receiving corticosteroids or immunosuppressive drugs.

(ii) *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.

(iii) *Preparation of target cells and lymphoid cells.* Target cells were chicken erythrocytes washed and labelled with ^{51}Cr (Calder *et al.*, 1973b, 1974). Lymphoid cells were separated from heparinized peripheral blood (Evans, 10 u/ml by density centrifugation on Ficoll–Triosil) (Calder *et al.*, 1973b, 1974). Adherent cells were removed by incubation on plastic medical flats (Falcon Ltd) for 1½ hr at 37°C and the remaining lymphoid cells were resuspended in growth medium (RPMI-1640 supplemented with heat-inactivated foetal bovine serum, 10% v/v), counted and used in the cytotoxic assay.

(iv) *Cytotoxic assay.* The assay of antibody-dependent cytotoxic activity of peripheral blood lymphoid cells was performed as previously described (Calder *et al.*, 1974). Briefly, cultures were set up in triplicate in LP_3 culture tube (Luckhams Ltd) each containing target cells (4×10^4) and lymphoid cells (3×10^5–8×10^3) in a volume of 400 μl of either growth medium alone or medium containing 10^{-4} diluted rabbit anti-chicken red blood cell antiserum. Four concentrations of lymphoid cells were assayed corresponding to lymphoid cell:target cell ratios of 5:1,2·5:1, 1·25:1 and 1·2. Cultures were incubated at 37°C in air–5% CO_2 for 18 hr after which they were centrifuged and an aliquot (200 μl) of supernatant removed for radioactive counting (Wallac automatic well-type counter). Cytotoxicity was expressed as a cytotoxic index.

(v) *Enumeration of T and B lymphocytes.* T lymphocytes were identified using the sheep red blood cell rosette method and B lymphocytes using indirect immunofluorescence (Urbaniak, Penhale & Irvine, 1973).

RESULTS

The antibody-dependent cytotoxic activity of peripheral blood lymphoid cells from thyroid patients and control subjects is shown in Figs 1–4. Total lymphocyte counts and the numbers of circulating T and B lymphocytes in the thyroid patients and control subjects are shown in Table 1.

DISCUSSION

The results presented in Figs 1–3 show that the antibody-dependent cytotoxic activity of lymphoid cells from patients with primary hypothyroidism, Hashimoto thyroiditis or thyrotoxicosis who are either newly diagnosed and untreated or have received therapy for ≤ 1 year is significantly elevated above normal control values. This increase was most marked in the group of patients with untreated thyrotoxicosis where statistical significance was reached at three of the four lymphoid to target cell ratios tested. Patients with primary hypothyroidism showed a significant increase in cytotoxic activity at two of the four ratios while patients with Hashimoto thyroiditis reached significance at only one ratio. This apparent lesser degree of elevation of cytotoxic activity in the Hashimoto patients is, however, most probably related to the smaller number in this group of patients; of the twenty-eight patients tested only eight were either newly diagnosed and untreated or had received therapy for ≤ 1 year (as compared to seventeen primary hypothyroids and twenty-three thyrotoxics).

The observed increase in antibody-dependent cytotoxic activity of lymphoid cells may reflect an

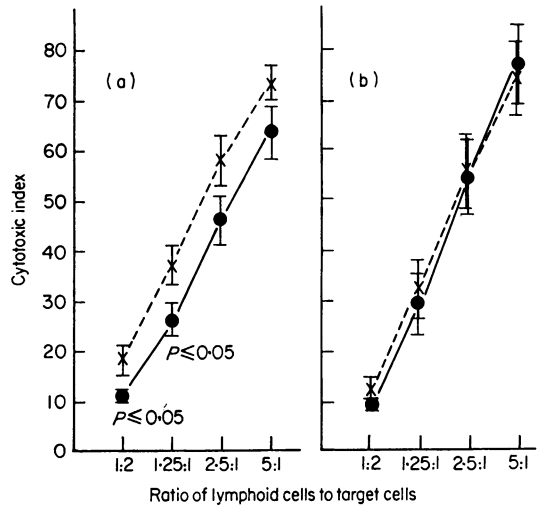

FIG. 1. K-cell cytotoxic activity of lymphoid cells from patients with primary hypothyroidism and from age- and sex-matched control subjects: (×) patients; (●) controls. (a) Patients either untreated or treated ≤1 year (n = 17); (b) patients treated >1 year and ≤5 years (n = 9).

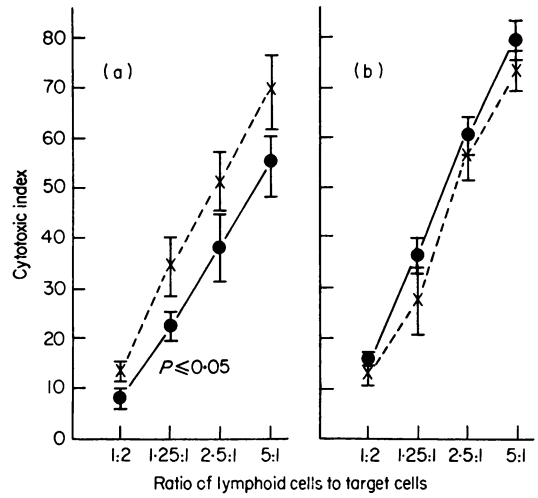

FIG. 2. K-cell cytotoxic activity of lymphoid cells from patients with Hashimoto thyroiditis and from age- and sex-matched control subjects: (×) patients; (●) controls. (a) Patients either untreated or treated ≤1 year (n = 8); (b) patients treated >1 year and ≤5 years (n = 20).

absolute increase in the number of circulating K cells or it may reflect a change in their cytotoxic capacity. For example, a patient with an absolute increase in K-cell numbers could still show cytotoxic activity within the normal range if the functional capacity of those cells was reduced, for example, by blocking of the Fc receptors by immune complexes. As far as an increase in cytotoxic activity is concerned, it is more logical to suppose that it does reflect an increase in K-cell numbers since it is difficult to see how the functional capacity of K cells could be actually increased.

It would therefore seem most likely that the elevated cytotoxic activity observed in untreated or short-term treated thyroid patients is due to an increase in the number of circulating K cells. After long-term treatment, K-cell cytotoxic activity falls back to within the normal range, possibly due to the re-establishment of the euthyroid state or to a reduction in the antigen load.

As shown in Fig. 4, within the group of patients with newly diagnosed untreated thyrotoxicosis, it was

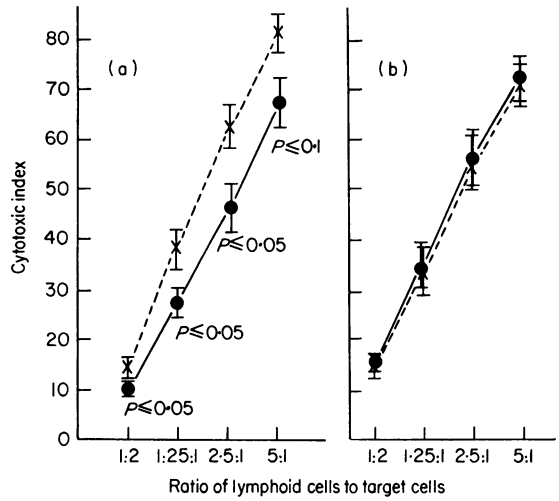

FIG. 3. K-cell cytotoxic activity of lymphoid cells from patients with thyrotoxicosis and from age- and sex-matched control subjects: (×) patients; (●) controls. (a) Patients newly diagnosed and untreated (n = 23); (b) patients treated with ^{131}I (n = 27).

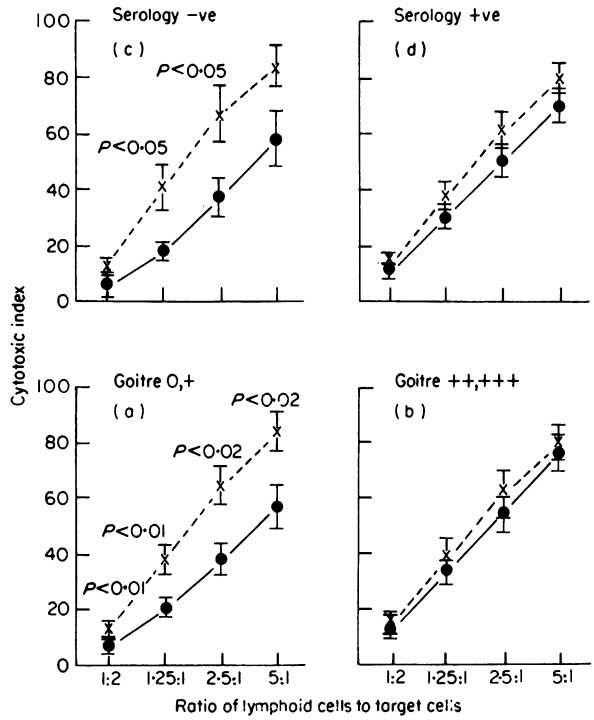

FIG. 4. K-cell cytotoxic activity in relation to goitre size and serum thyroid antibody titre in thyrotoxicosis: (×) patients; (●) controls. (a) Untreated thyrotoxics with a small or absent goitre (n = 10); (b) untreated thyrotoxics with a moderate to large goitre (n = 13); (c) untreated thyrotoxics with no detectable thyroid antibody (n = 6); (d) untreated thyrotoxics with positive thyroid antibody titres (n = 17).

found that K-cell cytotoxic activity was inversely related to goitre size and the titre of thyroid autoantibodies in the serum. Thus patients with a small or absent goitre showed a marked elevation of cytotoxic activity whereas those with moderate to large goitres gave values within the normal range. This

TABLE 1. Peripheral blood lymphocyte subpopulations in thyroid autoimmune diseases

		Total white blood count	Percentage lymphocyte	Total lymphocyte	Percentage T lymphocytes	Absolute T lymphocyte	Percentage B lymphocytes	Absolute B lymphocytes
Untreated thyrotoxicosis ($n = 21$)	Patient	6300±1489	31·2±9·0	1948±706	64·1±11	1241±443	19·7±6·3	395±214
	Control	6680±1898	28·9±8·8	1785±548	69·2±10	124·9±440	17·6±4·5	323±101
	2*P* value	>0·4	>0·3	>0·3	>0·5	>0·5	>0·2	>0·1
^{131}I treated thyrotocicosis ($n = 19$)	Patient	5980±1530	29·8±7	1763±500	58·4±12	1047±418	21·8±10	414±228
	Control	7080±2730	30·3±11	2050±800	63·7±10	1293±497	18·8±6·7	393±177
	2*P* Value	>0·1	>0·8	>0·1	>0·1	>0·1	>0·2	>0·7
Hashimoto's disease ($n = 19$)	Patient	5900±1680	29·8±9	1788±923	65·4±16	1184±693	20·3±6·5	357±175
	Control	6710±2340	27·9±9·6	1859±1011	67·2±8·8	1263±706	19±6	378±276
	2*P* Value	>0·2	>0·5	>0·8	>0·6	>0·7	>0·5	>0·3
Primary hypothyroidism ($n = 23$)	Patient	6817±1473	26±8·7	1602±630	54·3±12	855±367	19·2±8·5	311±223
	Control	6110±1520	28·6±7·2	1939±629	58·3±14·8	1113±470	18·7±7·6	356±130
	2*P* Value	>0·1	>0·2	>0·05	>0·3	>0·05	>0·8	>0·4

may be due to the sequestration of K-cells within the gland. Similarly, thyrotoxics with no detectable thyroid autoantibodies as detected by immunofluorescence and haemagglutination gave elevated K-cell cytotoxic activity while those patients with moderate to high titres gave values within the normal range. This finding could be related to a reduction in K-cell functional capacity. Patients with moderate to high titres of thyroid autoantibodies are likely to have higher levels of circulating immune complexes which could reduce K-cell cytotoxic activity. This interpretation gains support from concurrent studied (Barkas *et al.*, 1976) on the inhibitory activity of serum from patients with thyrotoxicosis on K-cell cytotoxicity where it was shown that sera from patients with moderate to high thyroid antibody titres were more inhibitory than sera from patients with negative thyroid antibody titres.

As summarized in Table 1, neither the absolute number nor the percentage of T and B lymphocytes in patients with Hashimoto thyroiditis, primary hypothyroidism and thyrotoxicosis was significantly different from that observed in age and sex-matched control subjects. This is in agreement with the findings of other workers (Urbaniak *et al.*, 1973, 1974; Wara *et al.*, 1973). The results of Farid *et al.* (1973), who reported an increase in the percentage of T lymphocytes in patients with Hashimoto thyroiditis and thyrotoxicosis, have recently been retracted (Volpé & Row, 1975).

In this study it was found that neither the total number nor the percentage of lymphocytes in patients with autoimmune thyroid disease was different from that observed in age and sex-matched control subjects. This aspect is being analysed in more detail and will be discussed in a later paper.

It was further found that the percentage of null cells [100−(percentage T+percentage B)] did not correlate with K-cell cytotoxic activity. This would suggest that the number of circulating K cells is unrelated to the number of null cells or alternatively that cytotoxic activity is not solely related to K-cell numbers. As discussed above, the functional activity of K cells could be reduced by blocking of Fc receptors by immune complexes. In future studies of K-cell cytotoxic activity it may prove useful to use lymphoid cells which have been freed of surface immunoglobulin components by enzyme treatment.

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