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# CELLULAR AUTOIMMUNE REACTIONS FOLLOWING RADIOIODINE TREATMENT FOR HYPERTHYROIDISM

by

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Radioiodine therapy is known to be followed by an increase in the circulating thyroid antibodies (BUCHANAN et coll. 1962, IRVINE 1964, O'GORMAN et coll. 1964, EINHORN et coll. 1965). In hyperthyroidism there may be an increase in antibodies to thyroid cytoplasmic antigen, though not to thyroglobulin (EINHORN et coll. 1965, JONSSON et coll. 1968), and in euthyroidism an increase in antibodies to both thyroid cytoplasmic antigen and thyroglobulin (EINHORN et coll. 1966). This increase in the circulating antibodies is temporary and lasts about a year after $^{131}I$ therapy (EINHORN et coll. 1965). A significantly higher frequency of positive serologic reactions has occurred in patients developing hypothyroidism within one year of $^{131}I$ treatment for hyperthyroidism (EINHORN et coll. 1965).

It is generally believed that the specific tissue damage occurring in the autoimmune states is mediated by cellular reactivity. It would therefore be of interest to know whether patients treated with radioiodine develop cellular reactivity to thyroid antigens, as happens in autoimmune states. This knowledge

Submitted for publication 9 June 1969.

might provide information on whether the autoimmune reaction induced by the local irradiation is of significance for the effect of radiation. The response of lymphocytes from such patients to thyroglobulin has been studied in vitro.

Thyroglobulin has been preferred to the microsome antigens in order to keep allotypic differences to a minimum. The stimulation of lymphocytes in vitro in the presence of antigen seems not to be closely dependent on circulating antibodies although in some way related to the state of delayed hypersensitivity (MILLS 1966, OPPENHEIM et coll. 1967). No definite proof has been presented, however, that such stimulation is a manifestation of delayed hypersensitivity.

During recent years several methods of examining lymphocytic reactions to antigens in vitro have been developed. One method relies on the fact that an increase in the incorporation of isotope-labelled thymidine by the cells indicates an increase in DNA synthesis in the presence of antigen (LING 1968). This method has been used in the experiments now reported. The stimulation of DNA synthesis by PPD tuberculin was tested on lymphocytes from subjects with positive skin reactivity to turberculin to verify that the method was adequate when an active stimulant was used. Lymphocytes and the thyroid glands of guinea pigs immunized with human thyroglobulin in complete adjuvant were studied in addition to lymphocytes from patients treated with radioiodine.

## Material and Methods

*Human lymphocyte donors.* The material consisted of 25 subjects divided into three groups comprising 11 healthy controls, 8 patients who had previously received radioiodine therapy and 6 patients with subacute thyroiditis.

The patients given radioiodine were all selected as having circulating antibodies to thyroglobulin or thyroid cytoplasmic antibodies at the time of examination. Their passive haemagglutination titres ranged from 1/80 to 1/12 500; four of them also had antibodies to cytoplasmic antigen as assessed by the fluorescent antibody (FA) technique. Seven of the patients were women and one was a man; their ages ranged from 49 to 69 years. They were examined 1—4 years after radioiodine treatment for hyperthyroidism, four of them having diffuse and four nodular goitres. The total dose of radioiodine ranged from 3.5 to 33 mCi $^{131}I$.

All the six patients with clinical signs of subacute thyroiditis had antibodies (FA) to microsome antigen, and four of them had haemagglutinating antibodies to thyroglobulin in titres ranging from 1/400 to 1/4 000 000. The five women and one man were from 29 to 43 years of age.

Eleven subjects, nine women and two men, aged between 24 and 48 years,

were taken as controls; none had demonstrable humoral antibodies to thyroid antigens.

*Experimental animals.* Nine male guinea pigs weighing about 400 g were immunized by injecting 2 mg of human thyroglobulin in complete Freund's adjuvant, as described for the immunization of this species with guinea pig thyroglobulin (WASSERMAN & PACKALÉN 1965). After 3 weeks the skin hypersensitivity to thyroglobulin was examined and the animals killed, the thyroid glands then being examined for histologic changes and the lymphocytes for stimulation of the DNA synthesis in the presence of thyroglobulin. Untreated male guinea pigs were used as controls.

*Methods.* Thyroglobulin was prepared from human thyroid tissue removed during surgery by the method described by LINDER & WEIBULL (1960). Several batches, each from one single gland, were used. PPD tuberculin (purified protein derivative) (kindly supplied by Parke Davis & Co, Detroit, Michigan) without preservatives was employed. One hundred milligrammes of phytohaemagglutinin were dissolved in 5 ml Hank-Tris buffer and frozen in small portions. About 50 to 100 ml of blood were withdrawn from a cubital vein of the patients and controls and defibrinated by cautious agitation with glass beads. The lymphocytes were isolated from the defibrinated blood by the method of COULSON & CHALMERS (1964). The cells were washed twice in Hank-Tris buffer, counted in a Bürker chamber and suspended in Eagle's medium supplemented with 10 % heat-inactivated autologous serum or fetal calf serum. One million lymphocytes were pipetted into conical tissue-culture tubes. The antigens in appropriate concentrations were added to the cells in a volume of 0.5 ml, or 250 to 500 mcg of PHA-M in a volume of 0.5 ml.

Tubes without antigens served as controls. The total volume of the incubation mixtures was 1.0 ml. The tubes were loosely closed with screw caps and incubated with a continuous flow of a mixture of 5 % carbon dioxide in air. After 72 hours, 0.4 $\mu$Ci of $^{14}$C-thymidine (specific activity 35.7 mCi/mM or 54.4 mCi/mM) was added to all tubes. After a further 24 hours the tubes were cooled in ice water and washed 4 times in cooled buffer. One drop of saturated $^{12}$C-thymidine was added to the first two washings. The washed cells were dissolved in 1.0 ml of concentrated formic acid and the liquid was transferred to planchets. After drying, the radioactivity was determined in a gasflow counter equipped with a Geiger-Müller tube. The uptake of $^{14}$C-thymidine in each sample was expressed in counts per minute, and the means of the values for duplicate tubes were taken. The results were expressed as the ratio between the counts per minute obtained with and without antigen. This ratio was called the 'lymphocyte stimulation index' (LSI).

Spleen cells were used in the guinea pig experiments in place of blood lymphocytes. The spleen was removed aseptically and cut into small pieces.

Suspensions of spleen cells were prepared by mincing the tissue in culture medium. In every other respect the method was the same as that used in the experiments with human blood lymphocytes. The stimulation tests were occasionally performed twice, with either autologous serum or fetal calf serum added to the culture medium. The results were the same for the two media.

When thyroglobulin was used as the antigen, 10, 100, 1 000 or 5 000 mcg were added to each tube in eight subjects; three of these concentrations of antigen were used in an additional thirteen subjects. In fourteen of these subjects the highest stimulation of the DNA synthesis was observed in the tubes to which 1 000 mcg thyroglobulin had been added, in three in the tubes to wich 100 mcg and in four subjects in the tubes to which 10 mcg had been added. The stimulation was never better with 5 000 than with 1 000 mcg; only one preparation with 1 000 mcg of thyroglobulin per tube was therefore tested. With PPD as antigen 5 mcg were added per tube.

Tubes with PHA-M were included as positive controls in each experiment. The phytohaemagglutinin always strongly stimulated DNA synthesis as measured by the uptake of $^{14}$C-thymidine.

Humoral antibodies to thyroglobulin and thyroid cytoplasmic antigens were tested at the National Bacteriologic Laboratory (JONSSON et coll. 1968).

The time after treatment was counted from the last radioiodine dose in patients given more than one dose of radioiodine.

## Results

The mean of the lymphocyte stimulation index in the healthy controls was 1.04 ± 0.11, in the patients receiving radioiodine therapy 1.45 ± 0.25 and in the patients with subacute thyroiditis it was 1.50 ± 0.23; the differences between these groups were not statistically significant. However, in the presence of thyroglobulin the lymphocyte stimulation index ranged in healthy donors from 0.62 to 1.57 (Table 1), and in the radioiodine-treated patients from 0.70 to 3.00. Three of the eight patients had an index higher than that for any of the eleven healthy controls (Ht3, Ht4, Ht5, Table 2).

The index ranged from 0.90 to 2.30 in subacute thyroiditis and in two of the six patients the value was higher than for any of the eleven healthy controls (T4 and T6, Table 3).

No correlation between the values of the lymphocyte stimulation index in the presence of thyroglobulin and of humoral antibodies to thyroglobulin, as tested by passive haemagglutination of tanned cells could be found.

**Table 1**

*Lymphocyte stimulation index (LSI) in the healthy controls — Mean of LSI = 1.04 ± 0.11 (standard error of the mean)*

| Subjects: | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| LSI | 1.57 | 1.37 | 0.39 | 1.09 | 0.92 | 1.40 | 0.62 | 0.86 | 0.71 | 1.41 | 1.05 |

**Table 2**

*Lymphocyte stimulation index (LSI) in the patients treated with $^{131}I$ for hyperthyroidism — Mean of LSI = 1.43±0.25 (standard error of the mean)*

| Subjects: | Ht1 | Ht2 | Ht3 | Ht4 | Ht5 | Ht6 | Ht7 | Ht8 |
|---|---|---|---|---|---|---|---|---|
| LSI | 1.14 | 1.09 | 1.63 | 1.69 | 3.00 | 1.30 | 0.70 | 0.90 |
| Years after radiotherapy | 1 | 3 | 1 | 3 | 4 | 3 | 2 | 3 |
| Total $^{131}I$ dose mCi | 33 | 22 | 12 | 3.5 | 22 | 6 | 5 | 3.5 |

**Table 3**

*Lymphocyte stimulation index (LSI) in Hashimoto thyroiditis — Mean of LSI = 1.50±0.23 (standard error of the mean)*

| Subjects: | T1 | T2 | T3 | T4 | T5 | T6 |
|---|---|---|---|---|---|---|
| LSI | 1.40 | 0.95 | 1.43 | 2.04 | 0.90 | 2.30 |

The addition of PPD tuberculin stimulated DNA synthesis in both lymphocyte donors with a lymphocyte stimulation index of 4.9 and 7.0; these controls gave positive skin tests to tuberculin in a dilution of 1: 1 000.

When the human thyroglobulin used in the above stimulation study was injected with complete Freund's adjuvant into the 9 guinea pigs, a delayed-type skin sensitivity to thyroglobulin developed. Stimulation of DNA synthesis in the presence of thyroglobulin was demonstrated in lymphocytes from most of the animals; morphologic signs of thyroiditis were observed in the four submitted to histologic examination.

## Discussion

It is evident from the stimulation of DNA synthesis by PPD tuberculin that the experimental system employed in this study was effective when the stimulant was active.

Immunization of the guinea pigs with human thyroglobulin in complete adjuvant produced the usual sequence of events (McMaster et coll. 1961, Wasserman & Packalén 1965); of particular interest was the development of thyroiditis. Delayed hypersensitivity is a specific immunologic manifestation induced by many heterologous antigens incorporated in complete adjuvant. The induction of thyroiditis points to an organ-specific activity of the thyroglobulin preparation used.

The results of the experiment with lymphocytes from patients with thyroiditis are in agreement with those of other workers. Only occasional positive results have been reported (Lycette & Pearmain 1965, Forbes 1965). De Groot & Jaksina (1969) in Hashimoto thyroiditis observed no stimulation of the thymidine incorporation in lymphocytes neither by thyroid supernatant fraction nor by thyroid microsomal fraction.

The main object of the present experiments was to ascertain whether lymphocytes from patients treated with radioiodine and displaying circulating antibodies to thyroid antigens were sensitized to thyroglobulin. The groups composing the material are small and the results preliminary. However, three of the eight patients receiving radioiodine therapy for hyperthyroidism had a higher lymphocyte stimulation index than any of the eleven healthy controls, and one recorded a value higher than the mean for the control group $\pm$ 5 times the standard error. This increase in the stimulation of blood lymphocytes to thyroglobulin in some of the radioiodine-treated patients may either be due to the effect of this local radiotherapy to the thyroid gland or may have been present before the radioiodine had been given. A considerable proportion of untreated patients with hyperthyroidism have demonstrable antibodies to thyroglobulin and to cytoplasmic antigens; no information is, however, available on the lymphocyte stimulation index in untreated hyperthyroidism (De Groot & Jaksina 1969).

It has previously been reported (Einhorn et coll. 1965, Jonsson et coll. 1968) that the increase in circulating thyroid antibodies after radioiodine therapy for hyperthyroidism is confined to antibodies to thyroid cytoplasmic antigens and is temporary, lasting about a year. There is no information as to whether the cellular reactivity in this respect follows the serologic reactivity. All the patients in this series had been treated with radioiodine more than a year previously.

## Acknowledgements

The authors take this opportunity of thanking Prof. Fagraeus as well as Drs Jonsson, Ljunggren, Almquist, and Wijnbladh for their cooperation in the work. The PPD tuberculin was supplied by Parke Davis, Detroit, Michigan and the phytohaemagglutinin by PHA-M, Difco Laboratories, Detroit, Michigan.

## SUMMARY

A material of 25 subjects, including eight patients who had previously received radioiodine therapy, was examined to determine whether cellular reactivity to thyroid antigens developed. The results, which are preliminary, are discussed in detail.

## ZUSAMMENFASSUNG

An einem Material von 25 Individuen einschliesslich acht Patienten, die vorher mit radioaktivem Jod behandelt worden waren, wurden Untersuchungen angestellt, um zu ermitteln, ob Zeichen einer zellulären Reaktion gegen Schilddrüsenantigene gefunden werden konnten. Die Resultate, die preliminär sind, werden diskutiert.

## RÉSUMÉ

Les auteurs ont cherché s'il apparaît une réactivité cellulaire aux antigènes thyroïdiens chez 25 sujets, dont huit malades avaient été traités par l'iode radio-actif pour hyperthyroïdie. Ils examinent en détail les résultats qui sont préliminaires.

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