FROM THE DEPARTMENT OF GYNAECOLOGY (DIRECTOR: PROF. H.-L. KOTTMEIER), RADIUMHEMMET, AND THE CENTRAL BACTERIOLOGIC LABORATORY OF STOCKHOLM CITY (DIRECTOR: PROF. T. PACKALÉN), STOCKHOLM, SWEDEN.

LYMPHOCYTE STIMULATION BY THYROGLOBULIN AFTER LOCAL IRRADIATION OF THE THYROID GLAND IN MAN

by

NINA EINHORN, T. PACKALÉN and J. WASSERMAN

Radioiodine therapy is known to be followed by an increase in the circulating thyroid antibodies (BUCHANAN et coll. 1962, O'GORMAN et coll. 1964, IRVINE 1964, EINHORN et coll. 1965). A rise in antibodies to the thyroid cystoplasmic antigens, but not to thyroglobulin, has been observed in hyperthyroidism (EINHORN et coll. 1965, JONSSON et coll. 1968) and an increase in antibodies to both thyroid cytoplasmic antigens and thyroglobulin in euthyroid subjects treated by radioiodine (EINHORN et coll. 1966). This increase is temporary and lasts about a year after the radiotherapy (EINHORN et coll. 1965). The fact that surgical treatment for hyperthyroidism has been followed not by an increase but by a significant decrease in thyroid antibodies (HJORT & MOGENSEN 1962, EINHORN et coll. 1965) indicates that the increase observed after radioiodine therapy was related to local irradiation and not induced by the reduction in size of the thyroid gland or in the basal metabolic rate. The increase in thyroid

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antibodies after local irradiation of the thyroid gland does not reflect a general rise in the autoimmune reactivity (JONSSON et coll. 1968). An increase in antibodies to the irradiated tissues has also been recorded after irradiation of the vagina and uterus by locally applied radium (EINHORN et coll. 1969). An increase in humoral antibodies to antigen present in the cell membrane of Burkitt's lymphoma and nasopharyngeal carcinoma was recently observed after radiotherapy in cases of these diseases in East Africa (EINHORN et coll. 1970b).

The tissue damage in autoimmune states is considered to be due more to the cellular reactivity than to the circulating antibodies. The response of lymphocytes to thyroglobulin was examined in vitro in patients with hyperthyroidism treated with radioactive iodine in order to ascertain the effect of local irradiation on the cell-mediated immunologic reactivity to the irradiated tissue. Thyroglobulin was chosen in preference to microsome antigen to keep allotypic differences to a minimum. It has previously been reported that in 3 out of 8 cases of hyperthyroidism treated by ^{131}I the reactivity of the lymphocytes to thyroglobulin was higher than in any of 11 controls (EINHORN et coll. 1970a).

Material and Methods

Lymphocyte donors. The material consisted of 46 unselected consecutive patients with hyperthyroidism admitted for radiotherapy; 37 were women and 9 men, ranging in age from 29 to 78 years.

The blood samples were obtained at regular clinical examinations to which the patients were submitted after radioiodine therapy. The first control was performed 6 weeks to 3 months after administration of the radioiodine and repeated usually every 3 to 4 months during the first year. A total of 180 successful tests were performed in these 46 patients.

Laboratory methods. The method for examining lymphocytic reactions to antigens in vitro relies on the fact that an increase in the incorporation of isotope-labelled thymidine by the cells reflects an increase in the DNA synthesis in the presence of antigen (LING 1968).

About 50 to 100 ml of blood drawn from a cubital vein of the patients and controls were defibrinated by careful agitation with glass beads. The lymphocytes were isolated ad modum COULSON & CHALMERS (1964). The cells were washed twice in Hank-Tris buffer, counted in a Burker chamber and suspended in Eagle's medium supplemented with 10 per cent of heat-inactivated AB serum. One or two million lymphocytes were pipetted into conical tissue-culture tubes in a volume of 0.5 ml. The antigens, in appropriate concentrations, were added to the cells in a volume of 0.5 ml.

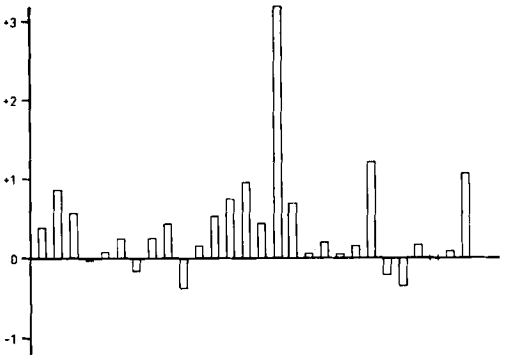
Thyroglobulin was prepared ad modum WEIBULL & LINDER (1960) from human thyroid tissue removed at operation; several batches, each from a single gland being used. Tubes without antigens served as controls; the total volume of the incubation mixtures was, however, always 1.0 ml. The tubes were loosely closed with screw caps and incubated with a continuous flow of a mixture of 5 per cent carbon dioxide in air. After 72 hours, 0.4 μCi of ^{14}C-thymidine was added to each tube and 24 hours later the tubes were cooled in ice water and washed twice in cooled buffer. One drop of saturated ^{12}C-thymidine was added to the first washing. The washed cells were treated with 5 per cent TCA, dissolved in 0.1 N sodium hydroxide and treated again with 6.7 per cent TCA. After drying, the pellets were dissolved in Soluene TM^{100} at 56° C. Scintillation fluid was added to the aliquots of the dissolved samples and the radioactivity measured in a liquid scintillation spectrometer. The uptake of ^{14}C-thymidine in each sample was expressed in counts per minute, and the means for duplicate tubes were taken. The results were discarded whenever the counts in duplicate incubations varied more than 35 per cent. The results were recorded as the ratio between the counts per minute obtained with and without antigen—the 'lymphocyte stimulation index' (LSI).

Amounts of 10, 100, 1 000 or 5 000 μg of thyroglobulin were added to each tube in preliminary experiments (EINHORN et coll. 1970). The highest stimulation of the DNA synthesis was observed usually in the tube to which 1 000 μg of thyroglobulin had been added, but the difference from the values for the 100 μg tubes was small and in 18 per cent of the cases these values were higher. Since, moreover, the stimulation was never better with 5 000 than with 1 000 μg, only preparations with 1 000 and 100 μg of thyroglobulin per tube were tested thereafter. In 40 cases the values used are those for the 1 000 μg tubes and in 6 cases—where at least one of the tests with the 1 000 μg tubes was unsuccessful—those for the tubes with 100 μg.

Statistical methods. The period after radiotherapy was divided into intervals corresponding as closely as possible to those for the routine clinical controls. The results of the test for each interval were, however, not available for all the patients; they had to be discarded in some tests because of the death of the lymphocytes or differences in counts in duplicate tubes, while in others the patients had not attended the routine examinations during the period prescribed for the investigation. The patients were not recalled simply for new samples.

The analysis included all the patients in whom the results of the test performed before the administration of the radioiodine and of at least one test after the radiotherapy were available.

The statistical analysis was carried out by the usual methods, including the Student's t-test. The difference in the index was obtained for each patient in



Lymphocyte stimulation index in the presence of thyroglobulin 6 weeks to 3 months after radioiodine therapy for hyperthyroidism as compared to the index before therapy. Each bar relates to one patient.

the comparison between the lymphocyte stimulation before and after the radioiodine therapy. The mean and the standard error of the mean were calculated for each series of these individual differences relating to each post-therapy period; it was then determined whether this mean was significantly different from 0.00.

Results

The geometric mean of the lymphocyte stimulation index for all the untreated patients with hyperthyroidism examined was 1.04; it did not differ significantly from 1.00. The index increased in 22, remained unchanged in 1 and decreased in 5 of the 28 patients tested before and 6 weeks to 3 months (42 to 91 days) after administration of the radioiodine (see the Figure); the mean was 1.01 ± 0.07 before and 1.43 ± 0.14 after radiotherapy. The increase of 0.42 ± 0.13 is statistically significant ($p < 0.01$). It seemed to be larger for the 16 patients examined within 75 days than for those examined at 75 to 91 days (0.52 ± 0.12 against 0.28 ± 0.14), but the groups were too small to justify any conclusions. In the patients examined within 75 days (42 to 75 days) the increase in the lymphocyte stimulation index was significant ($p < 0.05$). There was no significant increase in the lymphocyte stimulation in the patients examined 3 to 12 months after radiotherapy, but more than one year after radiotherapy there seemed again to be an increase in the lymphocyte stimulation. This increase was relatively small but statistically significant ($p < 0.05$; see the Table).

Discussion

An increase in thyroid antibodies after local irradiation of the thyroid gland is recognized (Buchanan et coll. 1962, O'Gorman et coll. 1964, Irvine 1964, Einhorn et coll. 1965). Local irradiation of the gland seems also to be followed

Table

Lymphocyte stimulation by thyroglobulin before and after ^{131}I therapy

Interval after ^{131}I therapy	Number of patients	Change in the LSI* after ^{131}I therapy; number of cases		Mean of individual LSI* differences		Level of significance for the difference, p
		Increase	Decrease	Mean	SE	
6 weeks—3 months	28	22	5	+0.42	0.13	<0.01
4—6 months	30	16	13	+0.01	0.11	—
7—9 months	22	14	8	+0.01	0.11	—
10—12 months	21	9	12	+0.09	0.14	—
⩾13 months	33	24	9	+0.23	0.10	<0.05

* Lymphocyte stimulation index.

by an increase in cellular reactivity, as reflected in the stimulability of the lymphocytes by a thyroid antigen, thyroglobulin.

The tests in this series were performed not earlier than 42 days after the administration of radioiodine. The period was chosen in the light of experience in an investigation of humoral antibodies in which an increase was present 2 to 12 months after radioiodine therapy (EINHORN et coll. 1965), but not before (EINHORN, FAGRAEUS & JONSSON, unpublished data). It is remarkable that the rise in the lymphocyte stimulation occurred early in the period, and it would be interesting to examine the reactivity of the lymphocytes even before 6 weeks after the supply of radioiodine. The reactivity of the lymphocytes to thyroglobulin was most marked 1.5 to 3 months after the irradiation; there was no significant increase 3 to 12 months after the radiotherapy. This does not exclude the possibility of increased cellular autoimmune reactivity having occurred during this period. Another and perhaps better method of examining cellular reactivity after local radiotherapy might be to test the delayed hypersensitivity to a thyroid antigen; such in vivo investigations in man are, however, complicated by the difficulty of preparing an antigen that entails no risk to the subject. Another promising approach might be to examine the lymphocytes in regional lymph nodes draining the irradiated tissues although this was not possible in the present material. The results now reported are, however, in line with previous publications on the relationship of cellular immunity to circulating antibodies. It is firmly established that cellular immunity and antibody production are due to distinct mechanisms. Most investigations on the induction of cellular immunity suggest that the latter may be demonstrated before any circulating antibodies appear (SZENBERG et coll. 1967). Research in guinea pigs with autoimmune thyroiditis

has revealed that cellular immunity to thyroglobulin appears before, and decreases earlier than, circulating antibodies (WASSERMAN et coll. 1965).

Although an increase in the humoral antibodies to thyroglobulin has been observed in euthyroid patients after radioiodine treatment (EINHORN et coll. 1966), such an increase in antibodies only to the cytoplasmic thyroid antigens (BUCHANAN et coll. 1962 and others) but not to the thyroglobulin (EINHORN et coll. 1965, JONSSON et coll. 1968) has been recorded in hyperthyroidism. A higher level of lymphocyte stimulation by thyroglobulin was apparent in hyperthyroid patients of the present material after radioiodine therapy.

Conclusions

An increase in the humoral antibodies occurs after local irradiation of the thyroid. In addition there is an increase in the cell-mediated reactivity to a thyroid antigen, as reflected in the lymphocyte stimulation by thyroglobulin. The rise in stimulability of peripheral lymphocytes seems to be of shorter duration than the increase in the humoral antibodies; the available results cannot however determine whether the increase in the stimulability of lymphocytes occurs first. While the rise in humoral antibodies was confined to the cytoplasmic antibodies and in patients with hyperthyroidism did not extend to the antibodies to thyroglobulin, a higher degree of stimulation by thyroglobulin was evident in the present material.

An increase in humoral antibodies also takes place after local irradiation of benign (EINHORN et coll. 1969) and malignant (EINHORN et coll. 1970), tissue other than the thyroid tissue, but whether this applies to the cellular reactivity as well is not known. Nor is it established whether the increase in stimulability of lymphocytes observed has a bearing on the biologic effect of the radiotherapy. Combined clinical and immunologic investigations in individual cases may provide further information.

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SUMMARY

Lymphocyte stimulation by thyroglobulin was investigated in 46 cases of hyperthyroidism before and after local irradiation of the thyroid gland by ^{131}I. A statistically significant increase in the stimulation occurred at six weeks to three months.

ZUSAMMENFASSUNG

Die Lymphozytenstimulation durch Thyreoglobulin wurde bei 37 Fällen von Hyperthyreoidismus vor und nach lokaler Bestrahlung der Thyreoidea durch ^{131}I untersucht. Ein statistisch signifikanter Anstieg der Stimulation erfolgte zwischen 6 Wochen bis zu 3 Monaten.

RÉSUMÉ

Les auteurs ont étudié la stimulation lymphocytaire par la thyroglobuline dans 46 cas d'hyperthyroïdie avant et après irradiation locale de la glande thyroïde par ^{131}I. La stimulation lymphocytaire est augmentée de façon stastiquement significative entre six semaines à trois mois.

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