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DNA-SYNTHESIS OF LYMPHOCYTES IN HYPERTHYROID AND EUTHYROID SUBJECTS

Effect of ^{131}I therapy on hyperthyroidism

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Treatment of hyperthyroidism with radioiodine is followed by an increase in circulating cytoplasmic thyroid antibodies (BUCHANAN et coll. 1962, O'GORMAN et coll. 1964, IRVINE 1964, EINHORN et coll. 1965, 1966, JONSSON et coll. 1968). Moreover, patients who develop hypothyroidism after such treatment have a significantly higher frequency of positive serologic reactions (EINHORN et coll. 1965, LUNDELL & JONSSON 1973). These findings motivated an investigation of possible changes in cellular immunity. The spontaneous DNA-synthesis of lymphocytes was evaluated by measurements of the incorporation of isotope labelled thymidine in vitro before and after ^{131}I therapy. This synthesis was considered to reflect at least to a certain extent the proliferative potential of the cells. In addition, the response of lymphocytes in the presence of thyroglobulin in vitro was examined.

It has been stated previously (EINHORN et coll. 1971) that ^{131}I treatment for hyperthyroidism is followed by an increase in lymphocyte reactivity to thyroglobulin. In the present report this statement is re-evaluated and the implications arising from the data presented are discussed.

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Material

The material consisted of three groups of patients and one group of controls.

Group A: 42 hyperthyroid patients, 36 females and 6 males, aged 29 to 78 years (mean 57 years), treated with ^{131}I during the period 1969 to 1970. Nineteen had diffuse and 23 nodular goitres and all belonged to a material presented previously (EINHORN et coll. 1971).

Group B: 19 patients, all female, aged 22 to 63 years (mean 34 years), treated by surgery for hyperthyroidism during 1970 and 1971.

Group C: 18 patients, all female, aged 29 to 62 years (mean 45 years), with atoxic nodular goitre treated by surgery during 1970 and 1971.

Group D (controls): 17 healthy volunteers receiving no treatment were examined by the same tests during the period of investigation. There were 14 females and 3 males aged 24 to 49 years (mean 37 years).

The patients given ^{131}I therapy were treated according to the principles described by LARSSON (1955) and BELING & EINHORN (1961); the determination of thyroid function was made by the methods outlined by LUNDELL & JONSSON (1973). The patients were examined at intervals of 2 to 4 months during the first year and thereafter every year until the termination of this investigation, the minimum follow-up time being 3 years. All patients treated for hyperthyroidism became euthyroid within this period.

Patients belonging to group B were given antithyroid drugs and thyroxine preoperatively. They were euthyroid at the time of surgery and at preoperative blood sampling. As a rule, bilateral subtotal thyroidectomy was performed in these patients. Postoperatively the patients with atoxic nodular goitre were given thyroxine routinely.

Methods

Blood samples for the determination of DNA-synthesis in lymphoid cells were obtained at the clinical examinations before and after therapy.

Venous blood for lymphocyte separation was defibrinated by gentle agitation in a beaker containing glass pearls. Separation of the nucleated cells was performed by the method of COULSEN & CHALMERS (1964). The number of lymphoid cells was determined in a Bürker chamber after crystal violet staining. The cell suspensions used for the experiments regularly contained a minimum of 60 per cent lymphocytes.

In 33 of the 42 patients in group A, 1×10^6 lymphoid cells were used at the beginning and since 1970, 2×10^6 cells. In the remaining 9 patients 2×10^6 cells were used in all tests. In experiments with patients belonging to groups B and C, 2×10^6 cells were used. Experiments with lymphocytes from individuals belonging to Group D were performed with 1×10^6 or 2×10^6 cells.

The lymphoid cells were washed twice in Hank-Tris buffer and suspended in Eagle's medium supplemented with 10 per cent heat-inactivated serum from AB

Table 1

Incorporation of ^{14}C thymidine by lymphoid cells in vitro before and after ^{131}I therapy for hyperthyroidism. The mean value for all 42 patients before therapy was 2405 ± 440 cpm

Interval after ^{131}I therapy	No. of pat.	No. of tests	Change in thymidine incorporation after therapy. No. of cases		Mean of individual differences in thymidine incorporation, cpm		Significance of the difference, p
			Increase	Decrease	Mean	SE	
6 weeks–3 months	30	32	9	21	−1 265	619	<0.05
4–6 months	31	34	12	19	−1 245	617	NS
7–12 months	35	47	14	21	−1 247	469	<0.05
13–18 months	33	35	13	20	−1 126	419	<0.05
19–24 months	31	34	11	20	− 623	500	NS
⩾25 months	22	22	4	18	−1 305	504	<0.05

Table 2

Incorporation of ^{14}C thymidine by lymphoid cells in vitro before and after ^{131}I therapy for hyperthyroidism. The mean value for all 9 patients before therapy was 4 981 ± 1 335 cpm

Interval after ^{131}I therapy	No. of pat.	No. of tests	Change in thymidine incorporation after therapy. No. of cases		Mean of individual differences in thymidine incorporation, cpm		Significance of the difference, p
			Increase	Decrease	Mean	SE	
6 weeks-3 months	6	8	1	5	−2 153	2 412	NS
4–6 months	6	7	0	6	−3 692	1 876	NS
7–12 months	6	9	0	6	−4 056	1 444	<0.05
13–18 months	4	4	0	4	−5 118	844	<0.01

positive Rh negative donors. One or two million cells, as indicated in the text, were pipetted into conical tissue-culture tubes. The total volume of the incubation mixture was 1 ml. The tubes were loosely closed with screw caps and incubated in a continuous flow of a mixture of 5 per cent CO_2 in air. After 72 hours, 0.4 μCi of ^{14}C thymidine (specific activity 54 mCi/mM, The Radiochemical Centre, Amersham, England) was added to all tubes. Twenty-four hours later the cultures were completed by centrifugation in the cold and the addition of trichloracetic acid. The amount of incorporated isotope was determined by a Packard scintillation counter. Duplicate cultures were performed and mean ^{14}C thymidine incorporation, expressed as counts per minute (cpm) and the SE were calculated. Moreover, mean values of individual differences between cpm obtained before and at different time intervals after therapy were calculated in all patient groups.

Table 3

Incorporation of ^{14}C thymidine by lymphoid cells in vitro before and after surgery for hyperthyroidism. All 19 patients pretreated with antithyroid drugs and thyroxin and euthyroid upon examination before surgery. At that time the mean value was 1 309 ± 219 cpm

Interval after surgery	No. of pat.	No. of tests	Change in thymidine incorporation after surgery. No. of cases		Mean of individual differences in thymidine incorporation, cpm		Significance of the difference, p
			Increase	Decrease	Mean	SE	
6 weeks-3 months	12	12	6	6	1 151	757	NS
4–6 months	14	14	8	6	250	491	NS
7–12 months	15	24	10	5	1 027	523	NS
13–18 months	11	12	6	5	686	865	NS
19–24 months	3	3	1	2	428	1 154	NS

Table 4

Incorporation of ^{14}C thymidine by lymphoid cells in vitro before and after surgery for atoxic nodular goitre. The mean value for all 18 patients before therapy was 1 536 ± 269 cpm

Interval after surgery	No. of pat.	No. of tests	Change in thymidine incorporation after surgery. No. of cases		Mean of individual differences in thymidine incorporation, cpm		Significance of the difference, p
			Increase	Decrease	Mean	SE	
6 weeks–3 months	13	13	11	2	896	266	<0.01
4–6 months	16	16	9	7	491	452	NS
7–12 months	14	22	10	4	959	463	NS
13–18 months	11	13	7	4	2 433	1 339	NS

Thyroglobulin was prepared from human thyroid tissue removed during surgery by the method of WEIBULL & LINDER (1960). Several batches, each from one single gland, were used. Thyroglobulin as an antigen was added as 100 or 1 000 μg aliquots to each tube. In the calculations, the value for 1 000 μg of thyroglobulin has been used throughout with the exception of those few cases in the series of 42 hyperthyroid patients where 100 μg was added. The difference in stimulatory effect was small and in some cases higher with 100 μg than with 1 000 μg of thyroglobulin, which is consistent with the report of EINHORN et coll. (1971). When calculating the stimulatory effect of thyroglobulin, the number of individual counts per minute for the spontaneous ^{14}C thymidine incorporation has been subtracted in order to estimate the effect of thyroglobulin on its own.

In some patients the thymidine incorporation was determined more than once

Table 5

Healthy and euthyroid controls. Incorporation of ^{14}C thymidine by lymphoid cells in vitro

Year of test	No. of cells	Mean value, cpm	SE	No. of controls
1969/1970	1×10^6	1 000	189	12
1969/1970	2×10^6	1 813	396	12
1971	2×10^6	1 381	600	6

Table 6

Incorporation of ^{14}C thymidine by lymphoid cells in vitro in healthy controls and in patients before surgery or ^{131}I therapy. 2×10^6 lymphoid cells

Group	Thyroid function at blood sampling	Planned treatment	No. of subjects	Mean value, cpm	SE	Significance of the difference to group A
A	Hyperthyroid	^{131}I	9	4 981	1 335	
B	Euthyroid	surgery	19	1 309	219	<0.001
C	Euthyroid	surgery	18	1 536	269	<0.01
D	Euthyroid	—	12	1 813	396	<0.05

within a specific time period. In such cases the arithmetic mean of these tests was used in the statistical calculations.

The statistical test used was the Student's t-test.

Results

Spontaneous DNA-synthesis. The mean value of the thymidine incorporation in lymphoid cells in group A decreased after therapy on all the occasions examined (Table 1) in comparison with the values of the same patient before therapy. This was observed despite the fact that in 33 of the 42 patients 1×10^6 lymphoid cells were used before therapy and 2×10^6 cells after therapy. The mean changes for the whole series were significant for the periods 6 weeks to 3 months ($p<0.05$), 7 to 12 months ($p<0.05$), 13 to 18 months ($p<0.05$) and more than 24 months after therapy ($p<0.05$) (Table 1). A significant decrease was also noted in the group of patients where 2×10^6 lymphoid cells were used throughout (Table 2).

In group B, no decrease was found in the DNA-synthesis as compared with the pre-treatment values (Table 3). These patients were, however, already euthyroid before surgery due to pre-treatment with antithyroid drugs and thyroxine. On the other hand, after surgery there was an increase in the DNA-synthesis of lymphocytes at each time interval studied. This increase, however, was not significant (Table 3). Similarly, after surgical treatment of atoxic nodular goitre, there was some increase

Table 7

Incorporation of ^{14}C thymidine by lymphoid cells in vitro in presence of thyroglobulin before and after ^{131}I therapy for hyperthyroidism. The mean value for all 42 patients before therapy was 358 ± 178 cpm, when the individual values obtained in the absence of thyroglobulin are subtracted

Interval after ^{131}I therapy	No. of pat.	No. of tests	Change in thymidine incorporation after therapy. No. of cases		Mean of individual differences in thymidine incorporation, cpm		Significance of the difference, p
			Increase	Decrease	Mean	SE	
6 weeks–3 months	20	21	13	7	33	222	NS
4–6 months	28	31	16	12	42	216	NS
7–12 months	35	46	20	15	−118	228	NS
13–18 months	33	35	19	14	10	253	NS
19–24 months	31	34	17	14	− 1	174	NS
⩾25 months	22	22	11	11	−109	286	NS

Table 8

Incorporation of ^{14}C thymidine of lymphoid cells in vitro in presence of thyroglobulin before and after surgery for hyperthyroidism. All 19 patients pretreated with antithyroid drugs and thyroxine and euthyroid upon examination before surgery. At that time the mean value was 257 ± 99 cpm, when the individual values obtained in the absence of thyroglobulin are subtracted

Interval after surgery	No. of pat.	No. of tests	Change in thymidine incorporation after surgery. No. of cases		Mean of individual differences in thymidine incorporation, cpm		Significance of the difference, p
			Increase	Decrease	Mean	SE	
6 weeks–3 months	12	12	9	3	1 112	603	NS
4–6 months	14	14	10	4	434	251	NS
7–12 months	15	24	12	3	621	377	NS
13–18 months	11	12	6	5	111	261	NS

in the incorporation of thymidine in the lymphocytes, which was significant ($p < 0.01$) during the period 6 weeks to 3 months after the operation (Table 4).

No significant change was found in the rate of DNA-synthesis in the 12 healthy, untreated controls during the period 1969 to 1971 (Table 5). The number of cpm was found to be increased when 2×10^6 cells were used compared to the number of cpm when 1×10^6 cells were used (Table 5).

The extent of thymidine incorporation using 2×10^6 cells was significantly higher in hyperthyroid patients from group A in the initial tests, i.e. before isotope therapy, than in euthyroid patients before surgery belonging to group B ($p < 0.05$) or C ($p < 0.05$), or in healthy controls ($p < 0.05$) (Table 6).

Table 9

Incorporation of ^{14}C thymidine of lymphoid cells in vitro in presence of thyroglobulin before and after surgery for atoxic nodular goitre. The mean value for all 18 patients before therapy was 363 ± 129 cpm, when the individual values obtained in the absence of thyroglobulin are subtracted

Interval after surgery	No. of pat.	No. of tests	Change in thymidine incorporation after surgery. No. of cases		Mean of individual differences in thymidine incorporation, cpm		Significance of the difference, p
			Increase	Decrease	Mean	SE	
6 weeks–3 months	13	13	10	3	322	277	NS
4–6 months	16	16	10	6	72	213	NS
7–12 months	14	22	6	8	86	255	NS
13–18 months	11	14	4	7	201	462	NS

Table 10

Healthy and euthyroid controls. Incorporation of ^{14}C thymidine by lymphoid cells in vitro with thyroglobulin added. The individual values obtained in the absence of thyroglobulin are subtracted

Year of test	Mean value, cpm	SE	No. of controls
1969/1970	196	168	12
1971	586	227	6

DNA-synthesis in the presence of thyroglobulin. In patients treated with ^{131}I for hyperthyroidism no significant changes were found in the DNA-synthesis when thyroglobulin was added as antigen compared with the pre-treatment values (Table 7). On the other hand, the DNA-synthesis in the presence of thyroglobulin in the groups treated by surgery was somewhat increased compared with the values before surgery; however, these differences were not statistically significant (Tables 8, 9). Similarly, the DNA-synthesis in the controls was slightly increased in 1971 compared to the period 1969/1970, but the differences were not statistically significant (Table 10).

Discussion

The results reveal that spontaneous DNA-synthesis in lymphocytes from patients with hyperthyroidism was higher than in lymphocytes from healthy controls, patients with atoxic goitre or hyperthyroid patients under treatment with antithyroid drugs. Furthermore, DNA-synthesis in the lymphocytes of hyperthyroid patients decreased after ^{131}I therapy.

The incorporation of thymidine by lymphoid cells in the presence of thyroglobulin was not affected by ^{131}I therapy for hyperthyroidism or by a reduction in the size

of the thyroid gland by surgery. These observations of thymidine incorporation in patients treated with ^{131}I for hyperthyroidism contradict previously published results (EINHORN et coll. 1971). However, in this latter report, the effect of ^{131}I treatment upon the lymphocyte stimulation by thyroglobulin was calculated as the lymphocyte stimulation index (LSI), i.e. the ratio between the counts per minute obtained with and without thyroglobulin. Since the spontaneous incorporation of thymidine by lymphocytes is decreased after ^{131}I therapy, the LSI will increase in the absence of any increased stimulation by thyroglobulin.

The reason for the increased spontaneous DNA-synthesis in lymphocytes from hyperthyroid patients on the 4th day of culture as compared with lymphocytes from euthyroid individuals is not known.

The spontaneous DNA-synthesis in cultured lymphocytes, sometimes also called background stimulation, is probably due to several different mechanisms. Some of the cells are activated in vivo. It is known from experiments that without the addition of antigen or polyclonal mitogens the rate of DNA-synthesis in lymphocytes cultured in vitro usually declines rather rapidly (HARRIS & LITTLETON 1966). However, on the 4th day of culture there certainly are some cells entering the proliferative phase that were initiated in vivo, possibly through the mediation of mitogenic factors generated in the culture. Different components of the culture medium can be either mildly stimulatory or cytotoxic to lymphocytes. The level of background activity is for example markedly dependent upon the serum used as supplement to the medium. Macromolecules or antibodies to antigenic sites on the surface of lymphocytes are known to exert an influence on the background DNA-synthesis (LING 1968). The difference in the rate of this synthesis between lymphocytes from hyperthyroid and euthyroid individuals suggests, however, that thyroid hormones exercise some influence on DNA-synthesis. This influence might be manifested directly through a stimulatory effect on lymphocytes or indirectly through an increase in the viability of cells cultured in vitro or by potentiation of some of the stimulatory mechanisms discussed.

Another explanation of the increased DNA-synthesis seen in the lymphocytes from hyperthyroid patients is the possibility that in these patients a shift in the proportion of T- and B-lymphocytes with an increased frequency of T-cells occurs. Although both T- and B-cells proliferate in response to antigens there is considerable evidence that in many situations DNA-synthesis in vitro is expressed primarily by the T-cells (GREAVES et coll. 1974). However, the relative frequency of B- and T-cells in hyperthyroid patients does not seem to differ from that found in healthy individuals (LUNDELL et coll. 1975).

Finally, it is also known that mononuclear phagocytic and adherent cells increase the proliferative response of lymphocytes to antigenic stimulation. However, no absolute or proportional increase of such cells was observed in hyperthyroid patients (LUNDELL 1975).

Further investigations are obviously required to elucidate the mechanisms involved

in the increase of spontaneous DNA-synthesis observed in lymphocytes from hyperthyroid patients. In vitro experiments on the stimulatory capacity of thyroid hormones are in progress.

The decrease in the spontaneous DNA-synthesis which follows ^{131}I treatment of hyperthyroid patients is presumably due to the reduced level of thyroid hormones with subsequent repercussions on one or several of the possible mechanisms that are responsible for regulating the rate of lymphocytic DNA-synthesis in these patients.

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SUMMARY

The DNA-synthesis of human lymphoid cells as estimated by the measurement of thymidine incorporation in vitro was investigated in healthy controls and in patients with various thyroid disorders before and after therapy. Hyperthyroid patients treated with ^{131}I and surgery (euthyroid at initial blood sampling before surgery), patients with atoxic nodular goitre treated by surgery and healthy untreated control individuals comprised the material. The synthesis of DNA in lymphocytes was higher in hyperthyroid patients in comparison with euthyroid individuals, and decreased subsequent to ^{131}I therapy in the hyperthyroid patients. No decrease was recorded in the other groups of patients. No evidence suggesting a change in the lymphocyte reactivity to thyroglobulin was found in any of the patient groups.

ZUSAMMENFASSUNG

Die DNA-Synthese menschlicher lymphoider Zellen, gemessen mit der Inkorporation von Thymidin in vitro, wurde bei gesunden Kontrollen und bei Patienten mit verschiedenen Störungen der Thyreoidea vor und nach der Therapie untersucht. Das Material bestand aus hyperthyroiden Patienten, die mit ^{131}I und Chirurgie (euthyroid zum Zeitpunkt der initialen Blutprobe vor der Chirurgie), Patienten mit einer atoxischen nodulären Struma, die durch Chirurgie behandelt wurden und gesunden unbehandelten Kontrollpersonen. Die DNA-Synthese der Lymphozyten war bei den hyperthyroiden Patienten im Vergleich zu euthyroiden Personen höher und fiel im Anschluss an die ^{131}I Therapie bei den hyperthyroiden Patienten. Kein Hinweis auf eine Änderung der Reaktivität der Lymphozyten gegenüber Thyroglobulin bei irgend einer der Patientgruppen wurde gefunden.

RÉSUMÉ

La synthèse de l'ADN des cellules lymphoïdes humaines, évaluée par la mesure de l'incorporation in vitro de la thymidine, a été étudiée chez des témoins sains et chez des malades atteints de diverses affections thyroïdiennes avant et après traitement. Ce matériel comprenait des malades hyperthyroïdiens traités par ^{131}I et par chirurgie (euthyroïdiens au prélèvement sanguin initial avant traitement chirurgical), des malades ayant un goître

nodulaire atoxique traité par chirurgie et des sujets témoins en bonne santé non traités. La synthèse de l'ADN dans les lymphocytes était plus importante chez les malades hyperthyroïdiens que chez les sujets euthyroïdiens et diminuait après le traitement par ^{131}I chez les malades hyperthyroïdiens. Il n'y a pas eu de diminution dans les autres groupes de malades. Dans aucun des groupes de malades on n'a trouvé de signe faisant penser que la réactivité des lymphocytes à la thyroglobuline était modifiée.

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