

Characterization of the natural killer cell activity in Hashimoto's and Graves' diseases

B. K. PEDERSEN,¹ U. FELDT-RASMUSSEN,² K. BECH,³ H. PERRILD,⁴ K. KLARLUND,¹ & M. HØIER-MADSEN⁵

¹Laboratory of Medical Immunology, TTA 7544, Rigshospitalet, ²Department of Medicine E, Frederiksberg Hospital, ³Department of Medicine, Hvidøre Hospital, ⁴Department of Medicine C, Bispebjerg Hospital, ⁵Autoimmune Laboratory, State Serum Institute, Copenhagen, Denmark

Natural killer (NK) cell activity and blood mononuclear cell subpopulations were characterized in patients with Hashimoto's thyroiditis ($n = 11$), Graves' disease ($n = 20$), non-toxic goitre ($n = 10$) and in normal controls ($n = 22$). NK cell activity against K 562 target cells and the capability of IFN- α , IL-2, and indomethacin to enhance NK cell activity *in vitro* did not differ significantly between the groups. The percentages of large granular lymphocytes, CD5+, CD4+, CD8+ and CD16+ cells were normal in patients with non-toxic goitre, Hashimoto's and Graves' diseases. There was no correlation between NK cell activities and TgAb, MAb and TSAb. Although NK cell activity is suppressed in several autoimmune diseases, NK cell function is normal in patients with autoimmune thyroid disorders.

Key words: Graves' disease; Hashimoto's thyroiditis; natural killer cells.

Accepted for publication 29 April 1989.

Natural killer (NK) cells are functionally identified by their ability to lyse a wide variety of targets. Morphologically they are identified as large granular lymphocytes (LGL) (18); antibodies reactive with Fc-IgG receptors, e.g. Leu 11 (CD16) identify essentially all NK cells (8). Interferon (IFN) (12) and interleukin 2 (IL-2) (11) enhance NK cell activity, while down-regulation of NK cells has been demonstrated with, e.g. certain prostaglandins (PG) (4).

Hashimoto's thyroiditis and Graves' disease are thought to be due to a basic immunologic defect; as yet the precise abnormality triggering the chain of events leading to antibody production, thyroid gland structural abnormalities, and resultant thyroid function perturbations has not been defined (5). Calder et al. (6) and Bogner et al. (3) observed enhanced NK cell activity in Hashimoto's and Graves'

disease patients, whereas Papic et al. (13) found decreased NK cell activity in Graves' disease, and Iwatani et al. (8) demonstrated decrease of peripheral LGL in Graves' disease. Sack et al. (16) and Pedersen et al. (15) demonstrated normal NK cytotoxicity against K562 target cells. As it is of great importance to determine whether NK cells play a role in the destruction of thyroid cells in autoimmune thyroid disease, we have extended this line of inquiry. We have further examined patients with Hashimoto's and Graves' diseases partly included in our previous publication (15).

We have characterized baseline NK cell activity and the ability of IFN, IL-2 and the prostaglandin-inhibitor, indomethacin, to enhance the cytotoxic activity of NK cell activity. Further, we determined the quantity of LGL and the proportion of CD5+, CD4+, CD8+ and CD16+ cells in patients with Hashimoto's thy-

roiditis, Graves' disease, non-toxic goitre and in controls. We have examined whether these immunological parameters correlate to the thyroid function.

PATIENTS AND METHODS

Patients

A total of 41 patients with various thyroid diseases and 22 healthy controls were studied. Patient characteristics are given in Table 1. The diagnosis of Hashimoto's thyroiditis was based on the presence of a characteristic goitre, positive antithyroglobulin and/or antimicrosomal antibodies, and evidence of thyroid failure with low thyroid hormones and high TSH. All patients, except three studied at diagnosis, were euthyroid on L-thyroxine. The patients with Graves' disease all had diffuse uptake on Tc-scintigraphy and clinical and biochemical evidence of hyperthyroidism. Eleven of the patients were studied at time of diagnosis while still untreated. The remaining nine were studied after in median 12 months (range 4–36) of treatment with antithyroid drugs (thiamazole 5 to 10 mg daily). The patients with non-toxic goitres were all euthyroid with a diffuse or multinodular scintigram, and all were un-

treated. The controls were euthyroid without a goitre and received no medication.

Thyroid function

Thyroid function was evaluated by measurement of serum concentrations of total thyroxine (T4), total triiodothyronine (T3), T3 uptake test (T3U) and thyrotropine (TSH) by radioimmunological methods previously described (8). Free T3 and T4 indices were calculated as total hormone concentrations multiplied by T3U. Presence of microsomal antibodies (MAb) was determined by immunofluorescence, and thyroglobulin antibodies (TgAb) by enzyme linked immunosorbent assay (9). Thyroid-stimulating immunoglobulins (TSAb) were measured by an adenylate cyclase assay using the human thyroid homogluate (1). The immunoglobulins were prepared from serum by ammonium sulphate precipitation followed by extensive dialysis (1). The activity was expressed in percentage of immunoglobulins from controls, and activity exceeding 110% indicated presence of TSAb (2).

Assay for determination of NK cell activity

NK cell activity of blood mononuclear cells was measured using K562 target cells in a ^{51}Cr

Table 1
Characteristics of patients with thyroid diseases. Median and ranges are given

	<i>n</i>	F/M	Age (years)	Free T4 index (arbitrary)	Free T3 index (units)	TSH mU l	No. with TgAb/MAb/TSAb	TSAb
Hashimoto's thyroiditis	11	10/1	60 (26–90)	5.9 (1.4–14.7)	84 (60–141)	3.7 (< 3–> 80)	8/11/6	112 (82–138)
Graves' disease (untreated)	11	10/1	61 (20–79)	25.1 (7.4–31.0)	780 (98–934)	< 3 (< 3–< 3)	3/7/11	136 (115–300)
Graves' disease (during treatment)	9	7/2	63 (27–70)	7.0 (5.1–8.8)	124 (70–219)	< 3 (< 3–7.5)	1/8/7	115 (89–308)
Non-toxic goitre	10	10/0	53 (35–78)	7.3 (5.5–10.1)	105 (73–140)	< 3 (< 3–5.0)	2/2/6	118 (62–162)
Controls	22	19/3	39 (25–79)	6.6 (3.5–8.0)	122 (85–156)	< 3 (< 3–3.9)	3/5	n.d.

Table 2
NK cell activity in patients with thyroid diseases and in controls

	NK	NK + Il-2	NK + IF	NK + indo
Hashimoto's thyroiditis	25.1 (14.7-44.7)	34.1 (11.6-49.7)	49.7 (20.2-59.6)	32.4 (9.9-50.4)
Graves' disease (untreated)	34.4 (5.7-51.4)	50.0 (10.8-62.9)	58.6 (27.3-65.7)	37.5 (15.8-65.0)
Graves' disease (treated)	21.8 (12.4-32.4)	27.9 (19.2-44.5)	47.0 (31.3-60.7)	28.0 (17.9-42.1)
Non-toxic goitre	37.1 (23.9-60.6)	45.3 (37.0-69.2)	57.9 (47.8-68.7)	42.6 (30.8-64.5)
Controls	34.7	41.2	56.5	37.5

release assay as previously described (14). The assay was carried out at three effector/target (E/T) cell ratios: 80/1, 40/1 and 20/1. The results obtained at the different E/T cell ratios did not differ qualitatively. Only the NK cell activity at E/T cell ratio 80/1 is given below.

Incubation of effector cells with IFN- α : 10 μ l of 10⁵ IU/ml of IFN- α were added to 990 μ l of 10⁶ effector cells/ml. IFN was kindly provided by Dr. Robert Jordal, The Blood Bank, Copenhagen County Hospital, Gentofte, Denmark. The cells were preincubated with IFN for 1 h at 37°C and IFN was also present during the incubation with target cells.

Incubation of effector cells with Il-2: Purified Il-2 was purchased from Boehringer Mannheim, W. Germany. This Il-2 was obtained from culture supernatants of pooled human T lymphocytes stimulated with PHA and purified by ammonium sulphate fractionation and DEAE column chromatography. Il-2 was stored in small aliquots at -20°C until use. Il-2 at a final concentration of 10% was preincubated with 10⁶ effector cells/ml for 1 h at 37°C. Il-2 was present during the incubation with target cells as well. The Il-2 preparation used in our experiments contained 300 IU/ml, measured as previously described (12).

Table 3
% LGL, % CD5+, % CD8+, % CD4+, and % CD16+ cells in patients with thyroid diseases and in controls

	% LGL	% CD5	% CD8	% CD4	% CD16
Hashimoto's thyroiditis	3.0 (1.5-10.5)	67 (54-76)	28 (10-36)	42 (27-54)	6 (4-9)
Graves' disease (untreated)	4.1 (2.3-8.9)	61 (44-77)	20 (10-30)	45 (24-67)	9 (6-20)
Graves' disease (treated)	3.8 (2.1-10.1)	57 (42-68)	18 (6-47)	43 (26-49)	8 (3-10)
Non-toxic goitre	3.9 (2.0-8.6)	58 (43-67)	21 (5-21)	42 (19-55)	10 (5-17)
Controls	4.3 (1.5-8.5)	57 (43-67)	18 (8-45)	38 (22-46)	7 (1-25)

Incubation of effector cells with indomethacin: Indomethacin (Confortid, Dumex) was dissolved in RPMI and preincubated with effector cells for 1 h at 37°C at a final concentration of 1 µg/ml.

Enumeration of large granular lymphocytes (LGL): Mononuclear cells were cytocentrifuged and stained with May-Grünwald stain as described (14). LGL were identified as medium to large-sized lymphocytes with a relatively high ratio of cytoplasm to nucleus, azurophilic cytoplasmic granules and often an indented nucleus. Monocytes were distinguished from LGL by their morphological appearance. The human percentage of LGL was blindly determined by two independent observers counting at least 200 lymphocytes.

Cell surface marker analysis: The phycoerythrin-conjugated monoclonal antibodies Leu1 (CD5), Leu2 (CD8), Leu3 (CD4) and Leu11 (CD16) (Beckton-Dickinson, CA, USA) were used. Labelled cells were analysed by flow cytometry using a fluorescence-activated cell sorter analyser (FACS, Beckton-Dickinson).

RESULTS

A total of 30 of the 44 patients with thyroid diseases were positive for TgAb, MAb and/or TSAb, as opposed to five of 22 controls (Table 1). There was no relation between absence or presence of TgAb or MAb and NK cell activity. Furthermore, no correlation existed between TSAb and NK cell function.

The NK cell activity in the different groups of patients with thyroid diseases was compared with the NK cell activity in normal healthy controls. The NK cell activity of patients with Hashimoto's disease and Graves' disease on anti-thyroid treatment, was lower than that of normal healthy controls or non-toxic goitre, but this difference was not significant (Table 2). IFN- α , Il-2 and indomethacin significantly enhanced NK cell activity in all groups tested ($P < 0.01$). Baseline NK cell activity and the NK cell activity enhanced by *in vitro* stimulation

with IFN, Il-2 or indomethacin did not differ significantly between the groups (Table 3). Further, the % LGL, % CD5, % CD4, % CD8 and % CD16 mononuclear cells did not differ significantly between the groups.

DISCUSSION

It seems well established that cytotoxic antibodies and antibody-dependent cell cytotoxicity against the thyroid gland is involved in the pathogenesis of autoimmune thyroiditis. Otherwise there are conflicting studies on NK cell activity in autoimmune thyroiditis (reviewed by Burman & Baker (5)). Reduced, elevated and normal NK cell activity have all been demonstrated in patients with Hashimoto's and Graves' diseases. In our study neither the function nor the quantity of NK cells in patients with Hashimoto's and Graves' disease differed significantly from that of normal healthy controls or non-toxic goitre.

Although a defective NK cell function has been found by many patients with systemic lupus erythematosus (17) and Sjögren's syndrome (14), an impaired NK function is thus not a consistent finding in patients with autoimmune disorders.

ACKNOWLEDGEMENTS

We thank Dr. V. Andersen for valuable discussions. The excellent technical assistance of Bodil Bossman, Kirsten Ankersø and the technical staff at the Isotope laboratory, Frederiksberg Hospital, is acknowledged. The work was supported by the Danish National Association against Rheumatic Diseases.

REFERENCES

1. Bech, K. & Madsen, S. H.: Thyroid adenylate cyclase stimulating immunoglobulins in thyroid diseases. *Clin. Endocrinol. (Oxf.)* 11, 47-58, 1979.
2. Bliddal, H., Bech, K., Johansen, K. & Nerup, J.: Thyroid stimulating immunoglobulins in insulin dependent diabetes mellitus. *Eur. J. Clin. Invest.* 14, 474-478, 1984.
3. Bogner, U. & Wall, Jr.: Antibody dependent cell mediated cytotoxicity against human thyroid cells in Hashimoto's disease. *Clin. Res.* 32, 262A (Abstract), 1984.
4. Brunda, M. J., Herberman, R. B. & Holden, T.: Inhibition of murine natural killer cell activity by prostaglandins. *J. Immunol.* 124, 2682-2687, 1980.

5. Burman, K. D. & Baker, J. R.: Immune mechanisms in Graves' disease. *Endocr. Rev.* 6, 183-232, 1985.
6. Calder, A. E., Penhale, W. J., McLeman, D., Barnes, E. W. & Irvine, W. J.: Lymphocyte-dependent antibody-mediated cytotoxicity in Hashimoto's thyroiditis. *Clin. Exp. Immunol.* 14, 153, 1973.
7. Høier-Madsen, M., Feldt-Rasmussen, U., Hegedus, L., Perrild, H. & Hansen, H. S.: Comparative measurement of serum thyroglobulin autoantibodies by radioprecipitation, ELISA or haemagglutination for routine use. *Acta Pathol. Microbiol. Immunol. Scand. Sect. C* 92, 377-382, 1984.
8. Iwatani, Y., Amino, N., Kabutomori, O., et al.: Decrease of peripheral large granular lymphocytes in Graves' disease. *Clin. Exp. Immunol.* 55, 239-244, 1984.
9. Kierkegaard, C., Bech, K., Bliddal, H., Danneskiold-Samsøe, B. & Feldt-Rasmussen, U.: Thyroid stimulating antibodies in rheumatoid arthritis: an in vitro phenomenon. *J. Endocrinol. Invest.* 10, 485-498, 1987.
10. Lancer, L. L., Le, A. M., Philips, J. H., Werner, L. L. & Bubcock, G.: Subpopulations of human natural killer cells defined by expression of the Leu7 (HNK-1) and Leu 11 (NK-15) antigens. *J. Immunol.* 131, 1789-1796, 1983.
11. Ortaldo, J. R., Gerard, J. P., Hendersson, L. E., Neubau, R. H. & Rabin, H.: Responsiveness of purified natural killer cells to pure interleukin 2 (IL-2). In Oppenheim J. J. & Rabin, H. (eds.): *Interleukins, lymphokines and cytokines*, pp. 63-68. Academic Press, New York, 1983.
12. Ortaldo, J. R., Mantovani, A., Hobbs, D., Rubinstein, M., Pestka, D. & Herberman, R. B.: Effects of several species of human leucocyte interferon cytotoxic activity of NK cells and monocytes. *Int. J. Cancer* 31, 285-289, 1983.
13. Papic, M., Stein-Stereilein, J., Zakarija, M., McKenzie, J. M., Guffee, J. & Fletcher, M. A.: Suppression of peripheral blood natural killer cell activity by excess thyroid hormone. *J. Clin. Invest.* 79, 404-408, 1987.
14. Pedersen, B. K., Oxholm, P., Manthorpe, R. & Andersen, V.: Interleukin 2 augmentation of the defective natural killer cell activity in patients with primary Sjögren's syndrome. *Clin. Exp. Immunol.* 63, 1-7, 1986.
15. Pedersen, B. K., Perrild, H., Feldt-Rasmussen, U., Christensen, T., Klarlund, K. & Hansen, J. M.: Suppressed natural killer cell activity in patients with euthyroid Graves' ophthalmopathy. *Autoimmunity* 1988.
16. Sack, J., Baker, J. R., Weetman, A. P., Wartofsky, L. & Burman, K. D.: Killer cell activity and antibody-dependent cell-mediated cytotoxicity are normal in Hashimoto's disease. *J. Clin. Endocrinol. Metabol.* 62, 1059-1064, 1986.
17. Sibbitt, W. L., Froelich, C. J. & Bankhurst, A. D.: Abnormal interferon modulation of natural cytotoxicity in systemic lupus erythematosus. Relation to suppressive serum factors. *Arthritis Rheum.* 26, 1452-1459, 1983.
18. Timonen, T. & Saksela, E.: Isolation of human natural killer cells by discontinuous gradient centrifugation. *J. Immunol. Methods* 30, 285-291, 1980.

Address:

Bente Klarlund Pedersen

Laboratory of Medical Immunology TTA 7544

Rigshospitalet

Tagensvej 20

DK-2200 Copenhagen N

Denmark