Neutrophil chemotaxis, random migration, and adherence in patients with hyperthyroidism

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Abstract. We have examined neutrophil adherence, chemotactic activity, and random migration in 35 hyperthyroid patients with Graves’ disease and 106 normal volunteers. No statistically significant differences were found between granulocyte adherence of 17 hyperthyroid subjects (67 ± 15.6%) and 81 healthy volunteers (63.1 ± 17%). In 5 thyrotoxic patients, impaired neutrophil adherence was found, which resolved when thyroid function returned to normal. The neutrophil chemotactic activity of 32 normal controls was 107.5 ± 21.4 cells, and the random migration 36 ± 15.5 cells. No statistically significant difference was demonstrated in 13 hyperthyroid patients who had a neutrophil chemotactic activity of 102 ± 14.6 cells and a random migration of 31.2 ± 13.2 cells. Defective chemotactic activity and random migration was found in 2 patients. Neutrophil functions returned to normal in one of the two subjects who were re-evaluated when thyroid function recovered. In summary, 14% of hyperthyroid patients had impaired leukocyte functions. However, severe pyogenic infections are quite rare in hyperthyroid patients, indicating that the observed alterations in function of phagocytic cells are not clinically important.

Thyroid hormones exert profound metabolic effects in virtually all tissues in the body through a variety of mechanisms. These effects include transport of amino acids and electrolytes from the extracellular space to the interior of the cell, synthesis or activation of specific enzyme proteins and enhancement of intracellular events, including translation and transcription (1).

It is not surprising, therefore, that thyroid hormones may exert important effects on polymorphonuclear functions which play a key role in defending against microbial pathogens (2-4). Studies have suggested that patients with thyrotoxicosis have specific defects in chemotaxis, random migration, and adherence (5, 6). We have therefore examined these functions in patients with hyperthyroidism and in healthy volunteers who served as controls.

Patients and Methods

Adhesiveness of purified polymorphonuclear leukocytes (PMN) was examined in 81 healthy volunteers (49 males, 32 females) and in 20 patients with hyperthyroidism caused by Graves’ disease (6 males, 14 females) prior to treatment. Chemotaxis and random migration were simultaneously tested in 32 normal controls (20 males, 12 females) and in 15 thyrotoxic patients with Graves’ disease (5 males, 10 females).

The diagnosis of thyrotoxicosis was based on classic clinical findings and confirmed with appropriate laboratory data, including free T₄ (FT₄) and total T₃ in all patients.

Standard commercial kits were utilized to measure the FT₄ (Diagnostic Products Corporation, Los Angeles, CA) and the T₃ (Amersham International, plc., UK). Patients had no diseases other than thyrotoxicosis when leukocyte functions were examined, and were without drug therapy.

Polymorphonuclear leukocyte isolation

Human purified PMN (98% PMN, with less than 1 platelet per 1000 cells) were isolated from heparinized venous blood. The blood samples were obtained by veni-
puncture from healthy volunteers who had given consent in accord with the Helsinki II Declaration.

After sedimentation in 6% dextran, the leukocyte-enriched plasma was layered on a Ficoll-Hyphaque gradient and centrifuged at 400 x g for 30 min at 4°C, as previously described (7). The supernatant fluid was discarded and the resulting pellet was subjected to hypotonic lysis for 20 sec, to free PMN of contaminating red cells.

The PMN were resuspended at a concentration of 1 x 10^6 PMN/l in autologous serum for adherence and at a concentration of 1 x 10^9/l in Medium-199 (Earle’s salt with L-glutamine, Biological Industries, Kibbutz Beth Haemek, Israel) for the chemotactic assay.

Adherence assay
Granulocyte adherence was tested as previously described (8,9), using spun nylon fibres as the adherent surface. A 15-mm column length of 70 mg nylon fibre was packed and adjusted into individual siliconized Pasteur pipettes. One ml of prewarmed (37°C) PMN suspension in autologous serum (1 x 10^6/l) was introduced on the top of the column in the prewarmed sterile Pasteur pipette and allowed to filter for 10 min by gravity only. The percentage of granulocytes adhering to the column was calculated by counting the PMN in the effluent suspension as compared with the count before passing through the column. All experiments were performed in triplicate.

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\frac{100\% - \text{PMN/l in effluent sample} \times 100}{\text{PMN/l in original sample}} = \% \text{ PMN adherence}
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Chemotaxis assay
A 48-well chemotactic microchamber (Neuro Probe, Inc, Bethesda, MD) was used to determine random migration and chemotaxis (10, 11). To the bottom wells was added the chemoattractant FMLP (N-formylmethionyl-leucylphenylalanine) at a concentration of 10^-7 mol/l. A polycarbonate filter sheet, without PVP coating, containing 3-μm holes (Nucleopore Corp, Pleasanton, CA) was placed on top of the wells in the bottom plate. The gasket and top plate were fixed in place and 1 x 10^8 PMN/ml were added to the upper wells. The assembly was incubated for 60 min at 37°C in humidified air. After incubation, the filter was wiped off and stained with May-Grumwald-Giemsa stain. The results were quantitated by counting the mean number of cells in five fields under light microscopy with a 40 x objective, using an optical grid at 10 x magnification. The chemotactic index was calculated by subtracting the random migration from the chemotactic activity. Experiments were performed in quadruplicate.

Statistics
Statistical analysis was performed using the Student’s t-test.

Results
The range of the FT₄ values in hyperthyroid subjects was 27.5—99 pmol/l with a mean ± 1 SD of 47.5 ± 16.3 pmol/l (normal range: 8.8—25 pmol/l). The range of T₃ concentrations was 2.9—9.62 nmol/l with a mean ± 1 SD of 5.4 ± 2.3 nmol/l (normal range 1.1—2.6 nmol/l).

Granulocyte adherence in 81 healthy volunteers was 63.1 ± 17% (range: 36—96), whereas in 17 hyperthyroid patients it was 67 ± 15.6% (range: 36—89%) (Fig. 1A). No statistically significant differences were present between these groups. In 3 patients the adherence was found to be impaired. After treatment, adherence was corrected from 31, 22 and 32% to 57, 57 and 54%, respectively. No statistically significant difference was demonstrated between males and females.

The chemotaxis, random migration, and the chemotactic index performed in 32 normal controls was 107.5 ± 21.4 cells (range: 84—163), 36 ± 15.5 cells (range: 14—56) and 71.5 ± 12.3 cells (range: 50—96), respectively (Fig. 1B).

In 2 of the 15 hyperthyroid patients, defective chemotaxis and random migration were found. Granulocyte adherence was normal in these patients. In only one of the patients, reassessment was possible after treatment. The chemotactic activity increased from 60 to 121 cells and the random migration from 11 to 36 cells. The remaining 13 hyperthyroid patients had a chemotactic activity of 102 ± 14.6 cells, a random migration of 31.2 ± 13.2 cells, and a chemotactic index of 70.7 ± 11.2 cells (Fig. 1B), not significantly different when compared with the control group. There was no clinical difference between thyrotoxic patients with normal or abnormal chemotaxis and adherence studies.

Discussion
Phagocytic cells provide important protection against a wide variety of microbial pathogens (2,3). Abnormalities in polymorphonuclear function may be associated with pyogenic infections (4, 12—14). Recently, it has been suggested that thyrotoxic patients may have defects in PMN function, particularly in chemotaxis, random migration, and adherence (5, 6).

The leukocyte functions which we examined in our study were normal in the majority of the pa-
Leukocyte functions in normal controls and hyperthyroid patients. The numbers 1–3 depict the results of normal controls (1), hyperthyroid subjects with normal leukocyte functions (2), and hyperthyroid patients with abnormal leukocyte functions (3). Panel A displays the results of polymorphonuclear leukocyte (PMN) adherence as percent of PMNs adhering to nylon fibres (vertical axis). The results on panel B are expressed as number of cells migrating (vertical axis on the right).

Abnormal leukocyte functions were demonstrated, however, in five of the hyperthyroid patients (14%). In three, a remarkable defect in PMN adhesiveness was demonstrated and in two additional patients abnormal chemotactic activity and random migration were found. Leukocyte dysfunctions resolved in the four patients who were re-evaluated when thyroid function recovered.

Hyperthyroidism in Graves' disease is considered to be a result of autoantibodies reacting with and activating the TSH receptors on thyroid cells (15). These thyroid-stimulating antibodies (TS-ab) activate adenylate cyclase in thyroid cells (16). Other autoantibodies, TSH-binding inhibition immunoglobulins (TBII) are characterized by their inhibition of the action of TSH (17). Receptors for porcine TSH have been described on human neutrophils (18). Weitzman et al. documented the presence of antineutrophil autoantibodies in Graves' disease which were displaced by bovine TSH (19). They suggested that these antineutrophil autoantibodies and TBII were identical and 'directed against the same or similar antigens expressed on more than one tissue'.

One may postulate the presence of an additional minor group of antineutrophil autoantibodies with TS-ab activity. This would explain the leukocyte defects found in our patients. It has been shown that elevated cellular levels of cAMP, or exogenous agents increasing intracellular levels of cAMP, inhibit PMN adhesiveness, phagocytosis, degranulation, chemotaxis, and oxidative metabolism of the cells (20, 21). Antineutrophil autoantibodies with TS-ab activity, comparable to TSH, would lead to elevation of intraleukocyte cAMP with resultant leukocyte dysfunction (22). The presence of leukocyte dysfunction, indeed, could be an indirect indication of the presence of TS-ab-like antineutrophil autoantibodies. Unfortunately, we were unable to check for the presence of antineutrophil autoantibodies or TS-ab in our subjects to corroborate this speculation.

Of note, in no patients with hyperthyroidism in this study was neutropenia or severe pyrogenic infections recorded, including those with defective leukocyte function. This further indicated that function of phagocytic cells is not significantly affected in hyperthyroid patients.
References


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