Preliminary evidence of immune function modulation by thyroid hormones in healthy men and women aged 55–70 years

Clare F Hodkinson[1], Ellen E A Simpson[2], John H Beattie[3], Jacqueline M O'Connor[1], David J Campbell[1], J J Strain[1] and **Julie M W Wallace[1]**

[1]Northern Ireland Centre for Food and Health (NICHE) and [2]School of Psychology, University of Ulster, Coleraine, Northern Ireland BT52 1SA, UK

[3]Rowett Research Institute, Bucksburn, Aberdeen, Scotland AB21 9SB, UK

(Correspondence should be addressed to J M W Wallace; Email: j.wallace@ulster.ac.uk)

Abstract

A reciprocal relationship between the endocrine and immune system has been demonstrated under pathophysiological conditions. However, few studies have assessed the relationship between thyroid hormones and immune function in apparently healthy individuals. Therefore, to clarify our understanding of normal physiological endocrine–immune interactions this study aimed to examine the interrelationships between thyroid hormones and immunity in healthy individuals. Total triiodothyronine (T_3), total thyroxine (T_4) and markers of immune status were assessed in 93 free-living and apparently healthy individuals aged 55–70 years. T_3 and T_4 concentrations were determined by commercially available kits. Immune status was assessed using flow cytometry and biochemical markers. Statistical analysis was performed by partial correlation, controlling for age. Thyroid hormone concentration was positively associated with markers of inflammation ($P \leq 0{\cdot}05$), natural killer-like T cells ($P \leq 0{\cdot}001$), expression of interleukin-6 (IL6) by activated monocytes ($P \leq 0{\cdot}05$); percentage expression of memory T-lymphocytes ($P \leq 0{\cdot}01$), memory T-helper lymphocytes ($P \leq 0{\cdot}05$) and memory T-cytotoxic lymphocytes ($P \leq 0{\cdot}05$), and higher IL2 receptor density on CD3+T-lymphocytes ($P \leq 0{\cdot}05$). Thyroid hormone concentration was inversely associated with early lymphocyte apoptosis ($P \leq 0{\cdot}05$) and the ratio of naïve- to memory T-cytotoxic lymphocytes ($P \leq 0{\cdot}05$). The current study provides preliminary evidence of a role for T_3 and T_4, within normal physiological ranges, in the maintenance of lymphocyte subpopulations, and in mediating the inflammatory response. In conclusion, these findings highlight the potential implications of altered thyroid function in older individuals and the importance of future research examining thyroid–immune interactions.

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Introduction

Bidirectional communication between the central nervous system and immune system is mediated by the endocrine system, via the hypothalamic–pituitary–adrenal (HPA) axis (Felten & Felten 1991, Bellinger *et al.* 2002). Evidence, predominantly from animal studies, also suggests a direct reciprocal relationship exists between the thyroid gland and the immune system (Fabris *et al.* 1995, Klein 2006). Thyroid hormones are involved in numerous physiological processes, such as development, growth and the metabolism of lipids and carbohydrates. While it is believed thyroid hormones are not critical to the development of a normal immune response, they are thought to be involved in the maintenance of immune function in response to environmental stimuli and stress-mediated immunosuppression (Davis 1998, Dorshkind & Horseman 2000, Dorshkind & Horsemanelson 2001). Total triiodothyronine (T_3) is able to modulate immune function via nuclear receptors (TRs) to regulate target genes, and by non-genomic interactions with membrane receptors independent of protein synthesis (Yen 2001, Csaba *et al.* 2005, Pallinger *et al.* 2005); the mechanisms that are speculated to underlie the aspects of immunity such as the homing of thymocytes to peripheral lymphoid organs (Ribeiro-Carvalho *et al.* 2007), and immune response to vaccination (Klecha *et al.* 2000). The prevalence of thyroid function disorders increases with age (Mariotti *et al.* 1995, Chiovato *et al.* 1997, Latrofa & Pinchera 2005), and hypothalamic–pituitary–thyroid (HPT) dysfunction and altered thyroid metabolism occurs even in apparently healthy older individuals (Mooradian 1995, Chakraborti *et al.* 1999, Magri *et al.* 2002). Given the relationship between thyroid and immune function, alterations in thyroid hormone concentration with age may contribute to age-related changes in immune function (Stulnig *et al.* 1995, McNerlan *et al.* 1999, Santagostino *et al.* 1999, Bisset *et al.* 2004).

Previous investigation of the relationship between thyroid hormones and immune functions has been predominantly restricted to a limited range of immune markers in clinical populations with thyroid function disorders (Palmblad *et al.*

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1981, Nakanishi *et al.* 1991, Covas *et al.* 1992, Mariotti *et al.* 1992, Kretowski *et al.* 1999, Bossowski *et al.* 2003). While these studies demonstrate that altered thyroid hormone concentration is associated with altered immunity, it is less clear whether these observations are directly due to the actions thyroid hormones or underlying autoimmunity. As age is a significant factor in the incidence of thyroid disorders, the current study aimed to determine if thyroid hormone concentrations, under normal physiological conditions in healthy older individuals, mirrors the thyroid–immune alterations observed in clinical populations. The use of a comprehensive range of phenotypic and functional immune markers was employed to provide a more complete assessment of potential immune modulation by thyroid hormones.

Material and Methods

Participants

Individuals aged 55–70 years were recruited through media coverage, and leaflets, as well as national and local organizations with members spanning this age group. Individuals were screened based on defined exclusion criteria, adapted from the SENIOR protocol (Ligthart *et al.* 1984) which included: body mass index (BMI; <20 and >33 kg/m^2); abnormal haematology, liver and kidney function tests; dietary habits (for e.g. vegetarians and vegans); depression (score ≥ 11 on the geriatric depression scale); dementia (score ≤ 24 on the Mini Mental State Examination); pre-menopausal women; no more than three drugs per day including the use of antidepressants, laxatives, hormonal replacement therapy or other immune modulating medication; diagnosis of or treatment for pathological diseases (cancer, diabetes, insufficient renal and hepatic performance, metabolic, malabsorption and chronic inflammatory pathologies); habitual use of vitamin and/or mineral supplements within the last 6 months; alcohol consumption (>30 g/day for men and >20 g/day for women); and smoking (>10 cigarettes, cigars or pipe/day). Of the individuals screened, 15% (147) were invited to participate. Ninety-three apparently healthy late-middle aged individuals (48 females and 45 males) aged 55–70 years completed the study. The University of Ulster Research Ethical Committee granted approval for the study. All volunteers gave written informed consent in accordance with the declaration of Helsinki.

Experimental protocol and sample collection

Following an overnight (>12 h) fast, participants were asked to attend the research centre at 0830 h on the study day. Anthropometric measurements were undertaken and blood was collected. Height and weight was measured and used to calculate BMI as body weight (kg) divided by height (m) squared. Fasting, venous blood (55 ml) was collected into vacutainers containing K_3EDTA and sodium-heparin and into serum separator tubes. K_3EDTA and sodium-heparin whole blood was used for determination of immune status on a FACSCalibur flow cytometer (BD Biosciences, Oxford, UK) within 4 h of collection. K_3EDTA anti-coagulated whole blood was also used to assess full blood profiles, which were conducted at the Causeway Laboratory, Causeway NHSS Trust, Coleraine, UK. Serum was separated and stored at -80 °C until analysis at the end of the study.

Table 1 Anti-human conjugated monoclonal antibodies used in FACS analysis of immune markers in healthy individuals, aged 55–70 years

	Marker	Clone	Conjugate
Antibody			
Simultest γ_1/γ_1	Negative control	X40, X40	FITC/PE
Intracellular γ_1	Negative control	X40	PE
Intracellular γ_2	Negative control	X39	FITC
CD3	Pan T lymphocyte	SK7	FITC and PE
CD14	Monocyte	MϕP9	FITC
CD19	B lymphocyte	SK7, SJ25C1	FITC and PE
Simultest CD3/CD16/CD56	NK and NKT cells	SK7, B73·1, MY31	FITC/PE/PE
CD25	Intermediately activated cells (IL-2R)	2A3	PE
CD3/anti-HLA-DR	Late-activated T lymphocytes	SK7, L243	FITC/PE
CD4	T-helper lymphocytes	SK3	PerCP
CD8	T-cytotoxic lymphocytes	SK1	PerCP
CD45RO	Memory cells	UCHL-1	PE
CD45RA	Naïve cells	L48	FITC
IL-1β	Interleukin-1β	AS10	PE
IL6	Interleukin-6	AS12	PE
Annexin V	Early cellular apoptosis	–	FITC
PI	Late cellular apoptosis/necrosis	–	–
PHAGOTEST *E. coli*	Phagocytic capacity/activity	–	FITC

FACS, fluorescence activated cell sorter; CD, cluster of differentiation; FITC, fluorescein; PE, phycoerythrin; NK, natural killer; HLA, human leukocyte antigen; DR, D- related; PerCP, peridinin chlorphyll protein; IL, interleukin; PI, propidium iodide. All products were supplied by BD BioSciences, Oxford, UK, with the exception of PHAGOTEST *Escherichia coli*, which was supplied by ORPEGEN Pharma, Heidelberg, Germany.

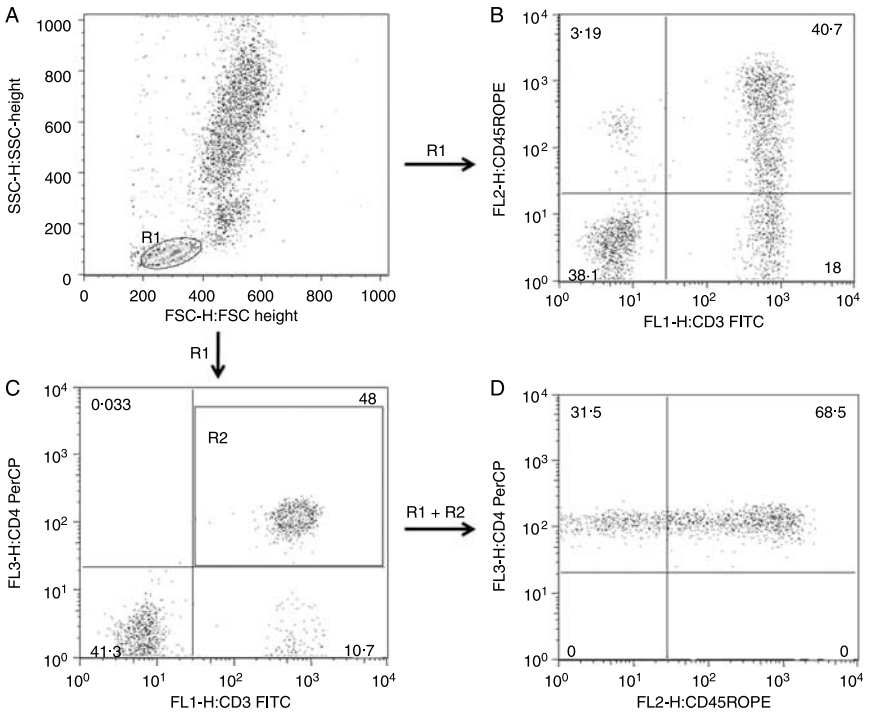

Figure 1 Gating strategy for the three-colour analysis of lymphocyte subsets. (A) Lymphocytes (R1) were identified by FSC versus SSC scatter characteristics excluding debris, monocytes and granulocytes. (B) Gating on R1 facilitated the determination of CD3+/CD45RA+ or CD3+/CD45RO+lymphocytes subsets. (C) CD3+/CD4+ (or CD3+/CD8+) lymphocytes were identified by gating on R1; a second gate (R2) was placed around CD3+CD4+ lymphocytes. (D) Gates R1+R2 enabled the determination of CD3+/CD4+/CD45RO+ or CD45RA+lymphocytes. The same gating strategy was applied for the determination of CD3+/CD8+lymphocyte subsets.

Thyroid hormones

Serum T_3 and total thyroxine (T_4) were measured using a competitive immunoassay with an enhanced chemiluminescence end point (Vitros Immunodiagnostic Products, Ortho-clinical Diagnostics, Amersham, UK).

Inflammatory markers

Serum was analysed for C-reactive protein (CRP-high sensitivity), complement protein-3 (C3), and complement protein-4 (C4), using commercially available kits on a Hitachi 912 analyzer (Roche Diagnostics). Serum ferritin was determined at Grenoble University Hospital by sensitized immunoprecipitation using automatic nephelometry (BNII nephelometer; Dade Behring, Paris La De'fence, France) as per the manufactures guidelines; laboratory quality assurance included analysis of serum from standard pools and international standards with each run.

Leukocyte immunophenotyping

Immunophenotyping of leukocyte subsets was carried out by two- and three-colour flow cytometry using panels of conjugated-monoclonal antibodies (mAb; Table 1). K_3EDTA whole blood (100 μl) was incubated with conjugated-mAbs using a lyse/wash protocol (BD Biosciences). Samples were analysed within 4 h of fixation. Two-colour analysis was performed for the following lymphocyte subsets: CD3+T-lymphocytes; CD3−/CD19+B-lymphocytes; CD3−/CD(16+56)+natural killer (NK) cells; CD3+/CD(16+56)+NK-like T (NKT) cells; CD3+/CD25+intermediately-activated T-lymphocytes; and CD3+/human leukocyte antigen-DR+ late-activated T-lymphocytes with the gating of lymphocytes (R1) using scatter characteristics (forward-scatter (FSC) versus side-scatter (SSC)) to facilitate the exclusion of monocytes and granulocytes from analysis. Three-colour analysis of CD3+/CD4+total T-helper lymphocytes, CD3+/CD8+total cytotoxic-T lymphocytes (CTL), CD3+/CD4+/CD45RA+naïve T-helper lymphocytes, CD3+/CD4+/CD45RO+memory T-helper lymphocytes, CD3+/CD8+/CD45RA+naïve CTL and CD3+/CD8+/CD45RO+memory CTL was performed by gating lymphocytes (R1) using scatter characteristics (FSC versus SSC) to facilitate the exclusion of monocytes and granulocytes from analysis, and either CD3+CD4+events (R2) or CD3+CD8+events to determine further CD45RO

or CD45RA T-lymphocyte subsets, see Fig. 1a–d. The percentage of positive cells was reported as obtained from fluorescence activated cell sorter (FACS) analysis and absolute counts were calculated from the lymphocyte white blood cell differential ($\times 10^9$/l). Ratios of lymphocyte subsets were calculated from absolute counts.

Apoptosis

The determination of early lymphocyte apoptosis by two-colour flow cytometry was conducted using the Annexin V-fluorescein (FITC) Apoptosis Detection Kit I (BD Biosciences). Briefly, peripheral blood mononuclear cells (PBMC) were separated by density gradient centrifugation from sodium-heparin anti-coagulated whole blood using Accuspin System-Histopaque-1077 tubes (Sigma–Aldrich). PBMC were washed twice in fresh sterile filtered PBS and then re-suspended in buffer. PBMC were stained using Annexin V-FITC and propidium iodide (PI). Lymphocytes were gated using a FSC versus SSC dotplot. Lymphocytes staining positive for Annexin-V and negative for PI were determined using fluorescence channel 1-height (FL1-H) versus fluorescence channel 2-height (FL2-H) dotplots (see Fig. 2a–c). The percentage of lymphocytes undergoing early apoptosis was obtained from FACS analysis, and absolute counts were calculated using the lymphocyte white blood cell differential ($\times 10^9$/l).

Phagocytosis

The quantification of phagocytic capacity and activity of granulocytes and monocytes was determined using a PHAGOTEST kit (ORPEGEN Pharma, Heidelberg, Germany). Sodium-heparin anti-coagulated whole blood (100 μl) was cooled on ice for 10 min, prior to incubation with opsonized *Escherichia coli*-FITC in capped Falcon tubes (BD Biosciences) for 10 min at 37 °C with 5–7% CO_2. Monocytes and granulocytes were gated using a FSC versus SSC dotplot. SSC versus *E. coli*-FITC dotplots were used to measure phagocytic capacity and activity for both cell types (see Fig. 3a–e). Percentage of *E. coli*-FITC-positive cells was used to determine the capacity of monocytes and granulocytes to conduct phagocytosis. Mean fluorescence intensity (MFI) was used as a quantitative measure to determine the number of *E. coli* ingested per cell; this measure was used as a determinant of phagocytic activity.

Determination of intracellular cytokine production by activated monocytes

To determine intracellular cytokine production by activated monocytes, 1 ml sodium-heparin anti-coagulated whole blood was activated by incubation with lipopolysaccharide at 1 μg/ml and secretion of intracellular cytokines blocked with brefeldin A at 10 μg/ml (Sigma–Aldrich) for 4 h at 37 °C with 5–7% CO_2. After activation, 100 μl activated blood was incubated with either 10 μl IgG2α FITC (isotype control) or CD14 FITC (BD Pharmingen, Oxford, UK) for 30 min in the dark at room temperature. Erythrocytes were lysed by incubation with 100 μl fixation medium A (Caltag, Invitrogen) for 30 min in the dark at room temperature. Cells were washed using cell wash solution (1 l sterile PBS containing 0·5% BSA and 0·1% NaN_3), and were centrifuged at 300 ***g*** for 5 min at 4 °C. Supernatant was removed, and the cell pellet re-suspended and incubated with 100 μl permeabilizing medium B (Caltag, Invitrogen) for 15 min in the dark at room temperature. Cells were then incubated with either (10 μl) of the isotype control IgG1-phycoerythrinphycoerythrin (PE), interleukin (IL1)β PE, or IL6 PE for 30 min in the dark at room temperature. Cells were washed as previously described, and fixed with 500 μl 1× Cell Fix solution (BD Biosciences). Samples were analyzed immediately.

Monocytes were gated using FSC versus SSC dotplots, and percentages of cytokine-positive CD14+ cells were obtained from FL1-H versus FL2-H dotplots (see Fig. 4a–c). Quantification of intracellular cytokine production, as determined by the antibody binding capacity, was achieved using PE QuantiBrite beads (BD Biosciences) for standardization of PE MFI.

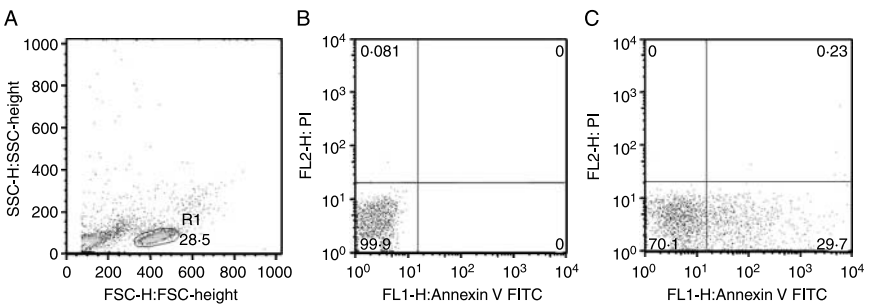

Figure 2 Gating strategy for two-colour analysis of early lymphocyte apoptosis. (A) Lymphocytes (R1) were identified by FSC versus SSC scatter characteristics. Dotplots B and C were gated on R1 and show the expression of Annexin V and PI for the unstained negative control (B) and stained (C) test sample.

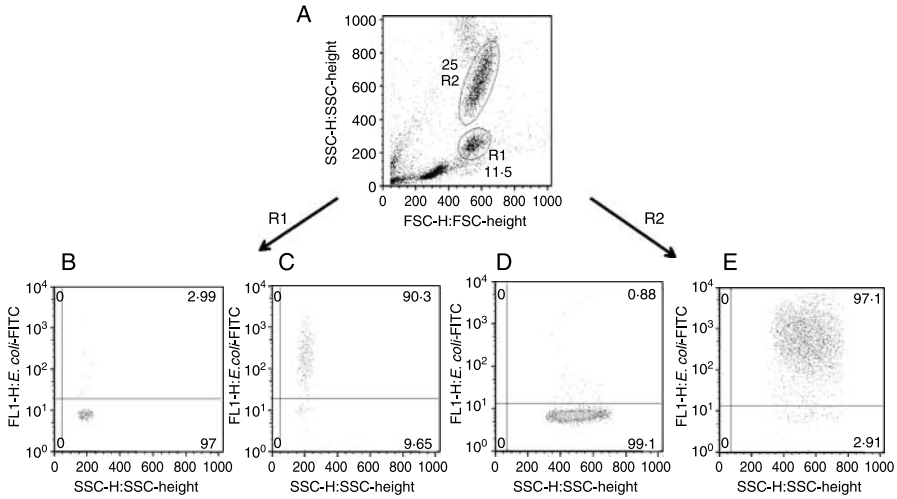

Figure 3 Gating strategy for one-colour analysis of phagocytic capacity and activity. The percentage of *E. coli*-FITC-positive cells was used to determine the capacity of monocytes or granulocytes to conduct phagocytosis. Mean fluorescence intensity (MFI) of *E. coli*-FITC-positive cells was used as a quantitative measure to determine the number of *E. coli* ingested per cell; this measure was used as a determinant of phagocytic activity. (A) FSC versus SSC scatter characteristics were used to identify monocyte (R1) and granulocyte (R2) populations. Expression of *E. coli*-FITC-positive R1-gated monocytes can be seen in control samples without *E. coli*-FITC incubation (B) and with incubation (C). Expression of *E. coli*-FITC-positive R2-gated granulocytes can be seen in control samples without *E. coli*-FITC incubation (D) and with incubation (E).

On each day of testing, three-colour calibration of the FACSCalibur was conducted using CaliBrite Beads-3 (BD Biosciences), and colour compensation levels using appropriate singly-stained cell suspensions. Flow cytometry data were acquired using CellQuest 3.3 for Power Macintosh (BD Biosciences) with 10 000 gated events analysed per sample. Cytometric data was analysed using FlowJo (version 4.5) for Macintosh (TreeStar, Ashland, OR, USA).

Statistical analysis

Statistical analysis was performed using a Statistical Package for Social Sciences (SPSS; Chicago, IL, USA) version 11.0. Data were transformed to approximate normal distribution where appropriate. Sex-differences in thyroid hormone concentration were assessed by independent *t*-test ($P \leq 0{\cdot}05$). As age was a significant determinant of immune function in this group (Hodkinson *et al.* 2006), the relationship between thyroid hormone concentration and immunity was explored using partial correlations, controlling for age.

Results

Participants

Participant characteristics are summarized in Table 2. At inclusion, volunteers were considered healthy and without incidence of infection in the preceding 4 weeks, as evidenced by self-reporting, a full blood profile, and normal liver and kidney function. Participants, were also unaffected by clinical depression and dementia. Participants had a mean age of 62·4 (S.D. 4·48) years and a mean BMI of 26·9 (S.D. 3·22) kg/m^2. There was no significant difference in age, BMI, T_3 or T_4 concentration between men and women.

Thyroid hormones

The euthyroid reference ranges for T_3 and T_4 were 1·49–2·60 nmol/l and 71·2–141 nmol/l respectively. Mean ($\pm$S.D.) values for the thyroid hormones T_3, T_4 and T_3:T_4 ratio were 1·95 ($\pm$0·30) nmol/l, 85·3 ($\pm$12·5) nmol/l and 0·23 ($\pm$0·003) ratio respectively, and were within normal range.

Markers of inflammation and immunity

Mean ($\pm$S.D.) serum CRP, C3 and C4 for all subjects were 0·20 ($\pm$0·36) mg/dl, 1·28 ($\pm$0·21) g/l and 0·26 ($\pm$0·06) g/l respectively, and were within normal ranges. Ten participants had subclinical inflammation, as indicated by elevated serum CRP concentration ($>$0·5 mg/dl). There was no significant difference in ferritin or immune markers between participants with normal and elevated CRP concentration, with the exception of monocyte count, which were higher in individuals with raised CRP concentration ($P=0{\cdot}014$). Medians (2·5–97·5 intervals) for all markers of immunity in this study population have previously been reported by Hodkinson *et al.* (2006).

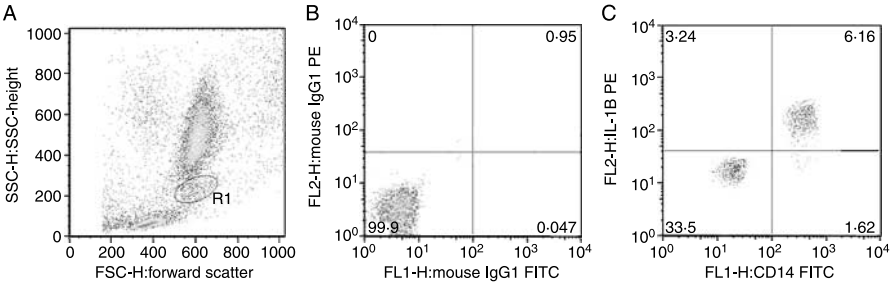

Figure 4 Gating strategy for two-colour analysis of lymphocyte intracellular cytokine expression and production by activated monocytes. Intracellular cytokine expression was determined from the percentage of IL-1β PE or IL6 PE positive monocytes. Quantification of intracellular cytokine production, as determined by the antibody binding capacity (ABC), was achieved using PE QuantiBrite beads for standardization of PE MFI. (A) Monocytes (R1) were identified by FSC versus SSC scatter characteristics. Dotplots B and C were gated on R1 and show the expression of CD14 and IL-1β monocytes for the isotype negative control (B) and stained (C) test sample. The same gating strategy was applied for intracellular IL6 expression and production by activated monocytes.

Associations between T_3, T_4 and markers of immunity

Studies examining immune–endocrine interactions involving the hormones T_3 and T_4 have assessed a limited range of immune markers, predominantly in individuals with thyroid disorders which may be confounded by the presence underlying autoimmunity. Therefore, in the current study T_3 and T_4 were measured in healthy adults in addition to a comprehensive range of phenotypic and functional markers of immunity. As age was a significant determinant of immune function in this group it was necessary to include age as a co-founder in all analyses to control for any influence on immune function. Calculation of mean T_3, T_4 concentration, and T_3:T_4 ratio was used to discriminate between low- and high-thyroid hormone concentrations within normal physiological ranges.

Table 3 shows the significant associations between thyroid hormones and markers of immune function. T_3 concentration was closely related to immune markers involved in innate immunity. Higher T_3 concentrations ($>1{\cdot}95$ nmol/l) were significantly associated with higher concentrations of complement proteins C3 ($P\leq0{\cdot}001$) and C4 ($P\leq0{\cdot}01$); increased monocyte phagocytic activity ($P\leq0{\cdot}05$); increased NKT percentage expression ($P\leq0{\cdot}001$) and absolute count ($P\leq0{\cdot}001$); and higher percentage expression of IL6 by activated monocytes ($P\leq0{\cdot}05$). In addition, T_3 concentration was positively associated with monocyte count, a finding that approached statistical significance ($P=0{\cdot}051$), and also demonstrated a significant inverse association with the percentage expression of early lymphocyte apoptosis ($P\leq0{\cdot}05$).

Table 2 Participant characteristics

	Males ($n=45$)	**Females** ($n=48$)
Age (years)	62·8±4·68	62·2±2·32
BMI (kg/m^2)	27·2±2·8	26·7±3·55
WBC ($\times10^9$/l)	5·48±1·28	5·10±1·09
T_3 (nmol/l)	1·89±0·22	1·99±0·36
T_4 (nmol/l)	85·6±11·9	85·0±13·1
T_3:T_4 ratio	0·022±0·003	0·024±0·003
Afternoon (1430 h) salivary cortisol (nmol/l)[a]	5·12±6·35	5·83±3·13
Evening (2200 h) salivary cortisol (nmol/l)[a]	4·63±10·4	3·35±5·61

All results mean±S.D.; *n*, number of participants; BMI, body mass index, WBC, white blood cell count.
[a]The number of participants included in the analyses were 21 males and 15 females. No significant differences were observed between male and female participants.

T_4 concentration was closely related to immune markers involved in both innate and adaptive immunity. Higher T_4 concentrations ($>85{\cdot}3$ nmol/l) were significantly associated with higher concentrations of complement proteins C3 ($P\leq0{\cdot}001$) and C4 ($P\leq0{\cdot}05$); higher CRP concentration ($P\leq0{\cdot}05$); and higher neutrophil counts ($P\leq0{\cdot}001$). T_4 was also positively correlated with the percentage expression of total memory T-lymphocytes ($P\leq0{\cdot}01$); the percentage expression of CD3+/CD4+/CD45RO+memory T-helper lymphocytes ($P\leq0{\cdot}05$); and the percentage expression of CD3+/CD8+/CD45RO+memory T-cytotoxic (CTL) lymphocytes ($P\leq0{\cdot}05$). In addition, T_4 concentration was inversely correlated with the ratio of naïve T-cytotoxic to memory T-cytotoxic lymphocytes ($P\leq0{\cdot}05$).

A higher T_3:T_4 ratio ($>0{\cdot}23$) was associated with increased monocyte phagocytic activity ($P\leq0{\cdot}05$); higher IL2 receptor density on CD3+T-lymphocytes ($P\leq0{\cdot}05$); and higher absolute counts of NKT cells ($P\leq0{\cdot}05$). Moreover, a higher T_3:T_4 ratio was also associated with lower neutrophil counts ($P\leq0{\cdot}05$).

Discussion

To our knowledge this is the first study to examine associations between thyroid hormone concentration and markers of immunity in healthy men and women, using a comprehensive panel of immune markers. Thyroid hormone

Table 3 Associations between serum thyroid hormones concentration and immune function in healthy individuals, aged 55–70 years[a]

			T_3 (nmol/l)	T_4 (nmol/l)	T_3:T_4 ratio
	n	**Mean±S.D.**	*r*	*r*	*r*
Immune parameter					
C3 (g/l)	89	1·28±0·21	0·375‡	0·329‡	0·004
C4 (g/l)	90	0·26±0·06	0·297†	0·254*	0·004
CRP (mg/dl)	90	0·20±0·36	0·173	0·208*	−0·083
Neutrophils ($\times10^9$/l)	90	2·87±0·87	0·151	0·398‡	−0·249*
Gated lymphocytes					
Annexin-V+/PI-Lymphocytes (%)	33	33·1±10·7	−0·382*	−0·170	−0·186
CD3+T-lymphocyte IL2 receptor density (MFI)	92	69·0±13·1	0·060	−0·167	0·215*
CD3+/CD16+/CD56+(%)	90	6·49±9·17	0·364‡	0·136	0·202
CD3+/CD16+/CD56+ ($\times10^9$/l)	90	0·12±0·22	0·333‡	0·095	0·212*
CD3+/CD45RO+(%)	88	51·1±13·2	0·181	0·286†	−0·097
Gated CD3+/CD4+ lymphocytes CD45RO+(%)	88	55·9±15·0	0·170	0·242*	−0·057
Gated CD3+/CD8+ lymphocytes CD45RO+(%)	87	45·9±16·7	0·105	0·272*	−0·164
CD45RA+:CD45RO+ ratio	82	1·53±1·14	−0·087	−0·224*	0·156
Gated monocytes					
Monocyte phagocytic activity (MFI)	89	234±112	0·227*	−0·035	0·231*
CD14+/IL-6+monocytes (%)	86	52·1±22·5	0·271*	0·052	0·180

n, number of participants; CD, cluster of differentiation. *$P\leq0·05$, †$P\leq0·01$, ‡$P\leq0·001$.
[a]As age was a significant determinant of immune function, age was used as a co-factor in all correlations between thyroid hormone concentration (total triiodothyronine, T_3; total thyroxine, T_4) and immune markers.

concentration and markers of immunity were within normal physiological ranges. Overall, the current study provides preliminary evidence to suggest that higher concentrations of T_3 and T_4, within normal physiological ranges, enhance innate and adaptive immunity through maintenance of specific cell populations and greater responsiveness to immune stimuli.

Our observation of a positive relationship between thyroid hormone concentration and markers of inflammation is supported by evidence for the binding of C4 to thyroperoxidase (TPO), a key enzyme required for thyroid hormone synthesis, that activates the complement cascade (Blanchin *et al.* 2003). Furthermore, *in vivo* clinical studies have indicated that altered thyroid hormone concentration is associated with serum IL6 concentration (Bartalena *et al.* 1994*a*,*b*). Furthermore, *ex vivo* studies have shown that thyroid hormones stimulate free-radical production in polymorphonuclear leukocytes (Mezosi *et al.* 2005), and that phagocytosis is impaired in hypothyroid patients (Palmblad *et al.* 1981). Our observations show that NKT cell numbers are associated with higher thyroid hormone concentrations within the normal physiological range. Altered NKT cell numbers and function are associated with increased incidence of autoimmune disorders (Miyake & Yamamura 2007), and increased NKT cell numbers have been observed in successfully aged individuals with normal thyroid function (Paolisso *et al.* 2000, Mocchegiani & Malavolta 2004, Abedin *et al.* 2005). However, studies in non-autoimmune thyroid disorders are necessary to clarify the relationship between altered thyroid hormone concentration and NKT biology.

Previous studies have reported elevated levels of soluble IL-2R in hyperthyroid patients (Nakanishi *et al.* 1991), and after oral T_3 administration (Mariotti *et al.* 1992). Our observation of increased surface IL-2R density on lymphocytes in healthy individuals with a higher T_3:T_4 ratio suggests that higher concentrations of the metabolically active thyroid hormone T_3, within normal physiological concentrations, may contribute to the expression of IL-2R on lymphocytes. T_3 was also associated with lower incidence of early lymphocyte apoptosis indicating that this thyroid hormone may also facilitate maintenance of the lymphocyte population. Although evidence for thyroid hormone regulation of lymphocyte responses has been demonstrated previously in animal models (Klecha *et al.* 2000, 2006), the underlying cellular mechanisms are not clearly understood.

The current study included an extensive panel of phenotypic markers of immunity that provided comprehensive information on immune status and, importantly, functional assays were included which are associated with clinical endpoints and also present a mechanistic understanding of the immune response (Albers *et al.* 2005). While, clearly as an observational study, the current study cannot discriminate cause-and-effect relationships between immune and endocrine function, it does highlight the requirement for further studies of these complex interactions in healthy individuals. Future studies should include a comprehensive assessment of immune status as described in the current study, but would benefit from the addition of further functional markers such as NK cell activity, phagocyte oxidative burst and the measurement of other pro- and anti-inflammatory

cytokines. To place the effects of T_3 and T_4 concentration on immune function in context free T_3 and T_4, TSH, antiTPO, antiTG, ACTH and lymphocyte TR assessment should also be incorporated into study designs to assess thyroid and adrenal gland function in relation to HPA- and HPT-axis activity.

In summary, the current study suggests a role for thyroid hormones, within the normal physiological range, in the maintenance of lymphocyte subpopulations, and in mediating the inflammatory response. This study suggests that some of the same immune alterations observed in thyroid disorders are also observed under normal physiological conditions, albeit within normal reference ranges. These preliminary findings indicate the potential benefit of maintaining optimal thyroid hormone concentrations for the maintenance of immune function with advancing age. Overall, the study reveals a complex relationship between the endocrine and immune systems that warrants further study in this vulnerable population of individuals.

Declaration of interest

The author's declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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