Thyroid hormone metabolism in innate immune cells

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Abstract
Thyroid hormone (TH) metabolism and thyroid status have been linked to various aspects of the immune response. There is extensive literature available on the effects of thyroid hormone on innate immune cells. However, only recently have authors begun to study the mechanisms behind these effects and the role of intracellular TH metabolism in innate immune cell function during inflammation. This review provides an overview of the molecular machinery of intracellular TH metabolism present in neutrophils, macrophages and dendritic cells and the role and effects of intracellular TH metabolism in these cells. Circulating TH levels have a profound effect on neutrophil, macrophage and dendritic cell function. In general, increased TH levels result in an amplification of the pro-inflammatory response of these cells. The mechanisms behind these effects include both genomic and non-genomic effects of TH. Besides a pro-inflammatory effect induced by extracellular TH, the cellular response to pro-inflammatory stimuli appears to be dependent on functional intracellular TH metabolism. This is illustrated by the fact that the deiodinase enzymes and in some cell types also thyroid hormone receptors appear to be crucial for adequate innate immune cell function. This overview of the literature suggests that TH metabolism plays an important role in the host defence against infection through the modulation of innate immune cell function.

Introduction
The interplay between the endocrine and immune system is well established (Besedovsky & del Rey 1996, Klein 2006, Schaefer & Klein 2011). Thyroid hormone (TH) metabolism and TH status have been linked to various aspects of the immune response (Boutzios & Kaltzas 2000, Klein 2006, De Vito et al. 2011), and there is an extensive body of literature available on the effects of TH on various types of innate immune cells (De Vito et al. 2011). However, very few of these studies analyse the mechanisms behind the effects of TH or the role of intracellular TH metabolism in innate immune cells. In recent years, the role of TH metabolism in innate immune cell function has been studied in more detail, and it has been suggested that innate immune cells are important T3 target cells and that intracellular TH plays an essential role in the function of several cell types of the innate immune system. This review provides an overview of the elements of intracellular TH metabolism present in innate immune cells and the role and effects of intracellular TH metabolism in these cells. It focuses specifically on the phagocytic innate immune cells: neutrophils, macrophages and dendritic cells.

Thyroid hormone production and metabolism
The regulation of plasma TH levels is conducted via a classic endocrine negative feedback loop involving
the hypothalamic–pituitary–thyroid (HPT) axis. Hypophysiotropic neurons within the paraventricular nucleus of the hypothalamus produce thyrotropin-releasing hormone (TRH), which in turn stimulates the thyrotroph cells of the anterior pituitary to synthesize and secrete thyroid-stimulating hormone (TSH) (Harris et al. 1978). TSH then stimulates the thyroid gland to produce thyroid hormones in the form of thyroxine (T₄) and triiodothyronine (T₃) (Miot et al. 2015). These hormones are secreted into the circulation, and their plasma levels in turn regulate the hypothalamic release of TRH, completing the feedback loop.

The thyroid gland mainly produces T₄, which functions as a prohormone and requires conversion to T₃ to become biologically active, although a minor role for direct actions of T₄ has also been reported (Davis et al. 2016). This conversion occurs at the cellular and tissue level, enabling the local regulation of TH bioavailability.

**Intracellular thyroid hormone metabolism**

TH is actively transported into the cell by TH transporters. There are several families of TH transporters including organic anion transporter polypeptides (OATP), monocarboxylate transporters (MCT) and large neutral amino acid transporters (LAT) (Bernal et al. 2015, Visser 2016). Of these transporters, MCT8 is the only one to transport TH exclusively. The other transporters are also capable of transporting additional substances including steroids and amino acids (Bernal et al. 2015). Studies on transgenic mouse models and observations in patients with pathogenic mutations in TH transporters indicate that MCT8, MCT10 and OATP1C1 are the main transporters of (patho)physiological importance to TH transport in vivo (Bernal et al. 2015, Visser 2016). MCT8 preferentially transports T₄, whereas MCT10 preferentially transports T₃ (Visser 2016). OATP1C1 transports T₄, T₃ and rT₃ with high specificity; however, it has the lowest affinity for T₃ out of these transporters (Pizzagalli et al. 2002, Bernal et al. 2015, Visser 2016). Transporter expression is cell type-specific and differences in distribution have been observed between humans and rodents (Bernal et al. 2015).

After being transported into the cell, TH is metabolized by the iodothyronine deiodinases. This a family of enzymes that remove an iodine atom from the phenolic or tyrosyl ring of TH (Bianco & Kim 2006, Visser & Peeters 2012). Type 1 deiodinase (D1) is capable of both inner and outer ring deiodination. Although it has a lower affinity for T₄ than the other deiodinases, it is highly expressed in the liver where it is thought to be the main source of local T₃ and to be important for the clearance of rT₃ (Bianco & Kim 2006). Type 2 deiodinase (D2) is capable of phenolic or outer ring deiodination resulting in the conversion of the prohormone T₄ to the active hormone T₃ (Bianco & Kim 2006). Approximately 80% of extra-thyroidal T₃ is derived from peripheral deiodination of T₄ mainly by D1 in the liver and D2 in skeletal muscle (Visser & Peeters 2012). Type 3 deiodinase (D3) is an inner ring deiodinase that converts T₄ and T₃ to their respective inactive metabolites rT₃ and T₂ (Bianco & Kim 2006).

Besides deiodination, there are other minor pathways of TH metabolism including sulfation, glucuronidation and ether-linked cleavage. The precise mechanisms of these metabolic pathways are beyond the scope of this review and have been discussed by other authors in more detail (Wu et al. 2005, Visser & Peeters 2012).

The classical pathway through which TH exerts its biological effects is by binding to the nuclear TH receptors (TRs). Upon binding of T₄, these TRs are capable of directly initiating or inhibiting gene transcription (Brent 2012, Mullur et al. 2014). There are several TR isoforms that are differentially expressed in a tissue- and cell type-specific manner (Brent 2012). The isoforms that are capable of binding T₃ are TRα1, which is widely expressed in cardiac and skeletal muscle, the central nervous system and bone, TRβ1, which is mainly present in the brain, liver and kidney and TRβ2, which is expressed in the hypothalamus and pituitary (Cheng et al. 2010, Brent 2012).

There is increasing evidence that THs also act via non-genomic pathways (Davis et al. 2016). The pathways involved in non-genomic TH actions are initiated by binding of TH to another receptor than the intracellular TRs, for example to the receptor on plasma membrane integrin αvβ3 (Davis et al. 2016). The classic pathways of TH action and the rapid non-genomic pathways activated by TH are not completely independent from each other as rapid non-genomic actions of TH can affect intracellular TRs and even require TRs in certain cell types (Davis et al. 2016, Flamant 2016).

**Innate immune cells**

The innate immune system is responsible for the host defence against invading pathogens. The cells of the innate immune system identify microbes, initiate an inflammatory response and can either directly phagocytose and kill pathogens or recruit other innate
or adaptive immune cells to the site of infection. Innate immune cells are derived from haematopoietic stem cells in the bone marrow. These cells can be mobilized from the blood or bone marrow upon infection. Alternatively, innate immune cells travel from the bone marrow to the tissue and patrol there for invading pathogens; these are known as tissue-resident cells. This review will focus on the phagocytic innate immune cells, which comprise neutrophils, monocytes/macrophages and dendritic cells.

Neutrophils

Neutrophils are the first cells to be recruited to the site of inflammation and are the most abundant type of blood leukocyte, comprising 50–75% of circulating leukocytes in humans (Borregaard 2010, Kolaczkowska & Kubes 2013, Bardoel et al. 2014). Circulating neutrophils are short-lived cells that are generated in the bone marrow by haematopoietic stem cells (Borregaard 2010).

Upon inflammation and infection, neutrophils from the circulation are recruited to the site of inflammation. Inflammatory mediators are recognised by neutrophils, after which they adhere to the vascular endothelium close to the site of infection before transmigrating into the extravascular tissue. Extravasated neutrophils then migrate to the place of inflammation where they can then kill invading pathogens and secrete inflammatory mediators further stimulating the immune response and recruiting other innate and adaptive immune cells (Kolaczkowska & Kubes 2013).

Neutrophils are highly specialized cells that have multiple microbial killing mechanisms at their disposal. These mechanisms have been discussed extensively in other reviews (Borregaard 2010, Kolaczkowska & Kubes 2013, Bardoel et al. 2014), therefore, we will only include a brief overview of these processes here. The three main killing mechanisms utilized by neutrophils are degranulation, the production of reactive oxygen species and the generation of neutrophil extracellular traps (Kolaczkowska & Kubes 2013, Bardoel et al. 2014). Upon phagocytosis of a pathogen, neutrophils can release various bactericidal elements into the phagosome. Some of these elements are antimicrobial proteins and enzymes that are formed sequentially during neutrophil development and stored in intracellular granules (Borregaard & Cowland 1997, Borregaard 2010). Upon phagocytosis, these granules can fuse with the phagosome or the plasma membrane, releasing their contents in a process known as degranulation (Borregaard 2010). Neutrophils are also capable of generating reactive oxygen species (ROS) in the phagosome using the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system. An important extracellular killing mechanism is neutrophil extracellular traps (NETs) (Brinkmann et al. 2004). NETs are composed of neutrophil chromatin to which antimicrobial proteins and ROS are bound (Brinkmann et al. 2004, Kolaczkowska & Kubes 2013). The release of these NETs enables neutrophils to effectively trap and kill extracellular bacteria, but also eventually results in the death of the neutrophil (Brinkmann et al. 2004).

Monocytes and macrophages

Monocytes and macrophages are mononuclear phagocytic cells. Monocytes are continuously generated in the bone marrow by haematopoiesis and released into the circulation where they constitute 10% of circulating human leukocytes. There is also a considerable monocyte reservoir in the spleen and lungs that can be mobilized on demand (Ginhoux & Jung 2014). Circulating monocytes can extravasate to tissues both during the steady state and during inflammation where they can differentiate into macrophages or dendritic cells (Shi & Pamer 2011). An alternative subset of macrophages is the tissue-resident macrophages. Until recently, these were thought to be continuously replenished from the circulating monocyte pool. Tissue-resident macrophages are now known to be derived from embryonic precursors that colonize the tissues prenatally (Mass et al. 2016). These cells, which include Kupffer cells and microglia, are also able to maintain their populations in adult tissues due to local cell proliferation independently of circulating monocytes (Hashimoto et al. 2013, Ginhoux & Jung 2014, Mass et al. 2016). The various tissue-resident macrophages comprise distinct cell populations whose phenotype differs strongly between tissues (Murray & Wynn 2011).

After entering the tissue, macrophages can change their phenotype due to various stimuli, allowing them to adapt to a wide subset of roles. This process is known as polarization. Polarized macrophages are generally classified into M1 or classically activated macrophages, which are pro-inflammatory cells, and M2 or alternatively activated macrophages, which is a heterogeneous group of cells that have a more anti-inflammatory profile (Murray & Wynn 2011). M1 macrophages are important in antimicrobial defence and the recruitment of neutrophils and T cells to the inflamed tissue (Murray & Wynn 2011). They are also capable of antigen presentation and can...
elicit a T-cell response (Hume 2008). M1 polarization is accompanied by changes in cellular metabolism, shifting towards enhanced glycolysis (Freemerman et al. 2014, Galvan-Pena & O’Neill 2014, Zhu et al. 2015). Essential components of adequate pro-inflammatory macrophage function are phagocytosis, the generation of ROS by NADPH oxidase and the generation of reactive nitrogen species (RNS), which is mediated by inducible nitric oxide synthase (iNOS) (Weiss & Schaible 2015). M2 macrophages are tolerogenic and immunomodulatory cells that are involved in wound healing and tissue remodelling (Murray & Wynn 2011). This is also accompanied by metabolic changes resulting in enhanced fatty acid oxidation and mitochondrial oxidative phosphorylation (Vats et al. 2006, Galvan-Pena & O’Neill 2014, Zhu et al. 2015). More recent data suggest that macrophage polarization is not as clear cut as these two phenotypes and represents more of a spectrum ranging from pro- to anti-inflammatory (Hume 2015).

**Dendritic cells**

Dendritic cells are unique innate immune cells that are not only capable of phagocytosing pathogens but also function as antigen-presenting cells. Dendritic cells thus bridge the gap between innate and adaptive immunity and can shape the T-cell response. The dendritic cell (DC) population is derived from the haematopoietic lineage and is more heterogeneous than previously thought. Currently four main types of DCs are recognised: classic DCs, plasmacytoid DCs, Langerhans cells and monocyte-derived DCs. All these subsets derive from a common myeloid progenitor (Satpathy et al. 2012, Pearce & Everts 2015). Classic DCs and monocyte-derived DCs are cells specialized in phagocytosis of pathogens. Unstimulated or immature classic DCs have a short half-life and are continuously replenished from precursors in the bone marrow (Satpathy et al. 2012). After activation these cells undergo considerable morphological changes and are characterized as mature classic DCs. Just as in macrophages, the activation of DCs is accompanied by a shift in cellular metabolism favouring glycolysis over oxidative phosphorylation (Pearce & Everts 2015). Mature DCs are capable of migrating to lymph nodes and subsequent antigen presentation to T cells, initiating and shaping the adaptive immune response. Plasmacytoid DCs are not phagocytic and inefficient at antigen presentation. They are thought to play an important role in the immune response to viruses as they produce large amounts of type 1 interferon upon viral encounter. Langerhans cells are tissue-resident DCs in the skin that resemble tissue-resident macrophages in many ways but are also capable of migrating to lymphoid tissues (Satpathy et al. 2012, Pearce & Everts 2015).

**Thyroid hormone metabolism in neutrophils**

**Intracellular thyroid hormone metabolism in neutrophils**

Neutrophils contain essential elements required for intracellular TH metabolism and action. Murine neutrophils contain the TH transporter MCT8, whereas human neutrophils express MCT10 but not MCT8 mRNA (Boelen et al. 2005, van der Spek et al. 2016). It has long been known that activated neutrophils are capable of deiodinating both T₃ and T₄ (Woebber 1971, 1978, Woebber et al. 1972, Klebanoff & Green 1973, Woebber & Ingbar 1973). Research from the seventies already found that phagocytosing human neutrophils can generate both T₃ and rT₃ from T₄ and that this deiodinating activity was mainly present in the granule fraction of the cells (Woebber 1976, 1978). Neutrophils were also shown to contain saturable nuclear-binding sites for T₃ (Woebber 1977). It has since been found that type 3 deiodinase (D3), the TH-inactivating enzyme, is present in both human and murine neutrophils and is located in the cytosol and in bactericidal granules within the cell (Boelen et al. 2005, 2008, van der Spek et al. 2016). Human neutrophils were also recently shown to express type 1 deiodinase (D1) and TRα1 at the transcriptional level (van der Spek et al. 2016).

**Effects of thyroid hormones on neutrophil function**

Neutrophil bacterial killing can be mediated by a number of different mechanisms one of which is the generation of reactive oxygen species (ROS) by the NADPH oxidase system (Kolaczkowska & Kubes 2013). Circulating TH levels affect ROS generation by stimulated neutrophils. Hyperthyroidism results in increased ROS generation by stimulated neutrophils *ex vivo* compared to cells from euthyroid controls, whereas hypothyroidism has the opposite effect and limits neutrophil ROS generation. This has been demonstrated in neutrophils from hypothyroid and hyperthyroid rats (Videla et al. 1993, Fernandez & Videla 1995) and hyperthyroid and hypothyroid patients (Videla et al. 1993, Szabo et al. 1996, Russo-Carbollante et al. 2005b, Marino et al. 2006) or healthy controls with experimentally induced hyperthyroidism (Magsino et al. 2000). In both hypothyroidism and...
Thyroid hormones and innate immune cells

Although studies using cells derived from hyperthyroid or hypothyroid patients and animals all find similar effects of TH levels on ROS generation, the effects of \textit{in vitro} incubation of neutrophils with TH are less consistent. Some authors find that \textit{in vitro} TH stimulation increases neutrophil ROS generation (Mezosi et al. 2005), whereas others only find an effect for supraphysiological levels of T$_4$ (Marino et al. 2006). In contrast, a decrease in ROS generation after TH incubation (Aoyagi et al. 1991) or no effect at all has also been reported (Videla et al. 1993). These conflicting results suggest that the effects of TH on neutrophil ROS generation cannot be entirely explained by direct effects of TH on these cells. The link between TH metabolism and oxidative stress that occurs outside innate immune cells has been reviewed elsewhere and is beyond the scope of this review (Mancini et al. 2016).

Another important neutrophil-killing mechanism is the use of antibacterial proteins housed in granules within the cell (Kolaczkowska & Kubes 2013). One of these proteins is myeloperoxidase (MPO). The only two studies to assess the effect of TH levels on MPO both find an increase in MPO activity in neutrophils either derived from hyperthyroid animals (Fernandez & Videla 1995) or incubated with TH \textit{in vitro} (Mezosi et al. 2005).

Circulating TH levels appear to have a clear effect on neutrophil function. Mezosi and coworkers found that these effects were mediated via non-genomic pathways. The increase in neutrophil ROS production elicited by TH incubation \textit{in vitro} was partially mediated via an unknown G-protein-coupled receptor and dependent on signalling through the protein kinase C pathway and increased intracellular Ca$^{2+}$ levels (Fig. 1) (Mezosi et al. 2005). Hyperthyroidism did not affect superoxide dismutase activity and glutathione content indicating that the increase in ROS generation found was not due to changes in antioxidant defences (Russo-Carbolante et al. 2005b).

**Figure 1**

Hypothetical pathways explaining the effects of thyroid hormone on neutrophil NAPDH oxidase activity and bacterial killing. Thyroid hormone induces neutrophil NADPH oxidase (NOX) activity, resulting in increased production of reactive oxygen species. This phenomenon is thought to be mediated via a non-genomic pathway involving binding of TH to a G-protein-coupled receptor (GPCR), which induces NAPDH oxidase activity. This effect is dependent on protein kinase C (PKC) and adequate intracellular Ca$^{2+}$ levels (Mezosi et al. 2005). Intracellular thyroid hormone metabolism may also play a role in neutrophils during bacterial killing. The thyroid hormone-inactivating type 3 deiodinase (D3) is present in murine and human neutrophils (Boelen et al. 2005, 2008, van der Spek et al. 2016). Mice that lack this enzyme suffer from impaired bacterial killing (Boelen et al. 2009). D3 is located in the cytoplasm and in granules containing either myeloperoxidase (MPO) or lactoferrin (LF) (van der Spek et al. 2016). TH enters the neutrophil via transporters (MCT8 or MCT10) where it is inactivated by D3, which removes an iodine atom from the inner ring of the hormone, converting T$_4$ to reverse (r)T$_3$ and T$_3$ to T$_2$. Increased D3 activity therefore results in decreased intracellular levels of T$_4$ together with the production of free iodide (I$^–$). One hypothesis explaining the role of D3 in microbial killing is that the iodide produced by D3 is utilized by MPO together with hydrogen peroxidase (H$_2$O$_2$) to generate hypiodide (IOH), a toxic compound that is capable of killing bacteria (Klebanoff 1967, Boelen et al. 2011). The reduction of intracellular T$_3$ levels could theoretically also result in altered gene transcription, but no TH-responsive genes have been found in neutrophils yet.

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Role of intracellular thyroid hormone metabolism in neutrophil function

Besides the effects of circulating TH levels on neutrophils, intracellular TH metabolism appears to play an essential role in neutrophil function during infection and inflammation.

THs are drawn to the site of bacterial infection (Adelberg et al. 1971). Activated phagocytosing neutrophils are capable of cleavage of thyroxine-binding globulin (TBG), thus increasing the amount of extracellularly available T₄ (Jirasakuldech et al. 2000). As mentioned previously, phagocytosing neutrophils also metabolize significant amounts of TH (Woebear 1971, 1978, Woebear et al. 1972, Klebanoff & Green 1973, Woebear & Ingbar 1973). This suggests that TH metabolism plays an important role in infiltrating neutrophils during infection.

Most authors find that metabolism of TH by phagocytosing neutrophils results in the production of inorganic iodide, suggesting the involvement of the deiodinase enzymes (Woebear et al. 1972, Klebanoff & Green 1973, Woebear & Ingbar 1973). Other authors have found that phagocytosing neutrophils are capable of ether-linked cleavage of T₃, which results in the formation of diiodotyrosine (DIT) (Burger et al. 1983). The degradation of TH requires the intracellular formation of ROS as neutrophils from patients with chronic granulomatous disease, which is characterized by defective NAPDH oxidase resulting in reduced ROS generation, have significantly impaired ability to degrade TH (Klebanoff & Green 1973, Woebear & Ingbar 1973, Burger et al. 1983). Although isolated MPO is capable of degrading TH in vitro, neutrophils from MPO-deficient patients degrade TH to the same degree as controls suggesting that the degradation of TH by leukocytes is not MPO dependent in vivo (Klebanoff & Green 1973, Woebear & Ingbar 1973, Burger et al. 1983).

Type 3 deiodinase (D3) is expressed in infiltrating murine neutrophils during both bacterial infection and sterile inflammation (Boelen et al. 2005, 2008). It was recently shown to also be present in human neutrophils (van der Spek et al. 2016). In a murine model for chronic local inflammation in which mice were injected with turpentine resulting in the formation of a subcutaneous abscess, D3 activity was strongly elevated in inflamed tissue compared to control tissue (Boelen et al. 2005). Mice that lack D3 have impaired bacterial killing upon infection with Streptococcus pneumoniae (Boelen et al. 2009). D3 in human neutrophils was found in intracellular granules involved in bacterial killing (van der Spek et al. 2016). The enzyme was also found in early-stage neutrophil extracellular traps (NETs) (van der Spek et al. 2016). Together these data suggest that D3 is important for neutrophil function during infection and inflammation.

Thyroid hormone metabolism in monocytes and macrophages

Intracellular thyroid hormone metabolism in macrophages

Macrophages contain several essential elements of intracellular TH metabolism. Both macrophage cell lines and human and murine microglia contain TH transporters. Macrophage cell lines predominantly express MCT10 and to a lesser extent MCT8 (Kwakkel et al. 2014). Microglia, the resident macrophages of the brain, contain the TH transporters LAT2, MCT10 and OATP4a1 (With et al. 2009, Braun et al. 2011). Macrophages were also found to express D2 (Kwakkel et al. 2014), TRα1 and possibly also TRβ although authors have reported conflicting results (Billon et al. 2014, Kwakkel et al. 2014, Lourboulos et al. 2014, Perrotta et al. 2014). Several recent papers have demonstrated that both human and murine macrophages are able to produce a functional TSHβ splice variant that is positively regulated by T₃ and capable of stimulating the TSH receptor (Vincent et al. 2009, Baliram et al. 2013, 2016). It has been suggested that this TSHβ splice variant plays a role in bone physiology; however, whether it affects macrophage function is currently unknown.

Effects of extracellular thyroid hormone levels on macrophage function

There are several studies available on the effects of TH administration on macrophage function either in vivo,
ex vivo or in vitro. The majority of these functional studies assessed pro-inflammatory macrophage function.

Several studies in both human subjects and animal models have found that, similar to neutrophils, stimulated hyperthyroid macrophages have increased ROS production ex vivo (Videla et al. 1993, Magsino et al. 2000) although one study found an opposite effect (Rosa et al. 1995). PTU treatment of hyperthyroid patients normalized ROS production although these findings could not be replicated in vitro (Videla et al. 1993). It should be noted that the majority of these studies assessed a mixed population of mononuclear cells containing significant numbers of other cell types, making it impossible to

determine the contribution of the macrophage/monocyte subset. Recently, TH administration was found to increase iNOS expression, nitrite production and in vitro bacterial killing in both a human and a mouse macrophage cell line and treatment with TH increased the survival after meningococcal infection in mice (Chen et al. 2012).

Macrophage phagocytosis is also affected by TH concentrations with most studies finding that higher levels of available TH result in an increased phagocytic capacity. Strenuous exercise leads to both increased plasma TH levels and increased macrophage phagocytosis (Forner et al. 1996, Ortega et al. 1996). This effect was confirmed in vitro where incubation of

Figure 2
Thyroid hormone induces a pro-inflammatory response in macrophages. (A) The effects of TH in macrophages are mediated through integrin αvβ3 or through modulation of intracellular TH levels. TH can bind to integrin αvβ3 on the macrophage cell surface resulting in the activation of PI3K and ERK1/2 pathways followed by the upregulation of inducible nitric oxide synthase (iNOS) (Chen et al. 2012). Alternatively, TH can enter the cell through TH transporters MCT8 or MCT10 after which the prohormone T4 is converted to active hormone T3 by type 2 deiodinase (D2) resulting in increased phagocytosis and cytokine response. D2 is induced in lipopolysaccharide (LPS)-stimulated macrophages (Kwakkel et al. 2014). The effects of intracellular T3 are partly mediated via TRα, which is required for adequate macrophage function (Billon et al. 2014, Kwakkel et al. 2014). Low-grade inflammation found in unstimulated TRα-knockout macrophages suggests that TRα possibly attenuates the rapid pro-inflammatory response generated by increased intracellular TH levels (Billon et al. 2014). (B) Kupffer cells are the resident macrophages of the liver. TH stimulation in vivo results in Kupffer cell hyperplasia and enhanced phagocytosis (Tapia et al. 1997, Valencia et al. 2004). TH transporter and receptor expression in Kupffer cells have not yet been studied. TH also increases the production of TNFα by Kupffer cells (Valencia et al. 2004, Fernandez et al. 2005, 2007b). TNFα produced by Kupffer cells results in liver NFκB activation (Valencia et al. 2004). IL-6 production is also increased, resulting in increased STAT3 activation (Tapia et al. 2006). The activation of both these pathways results in increased iNOS activity in the liver, resulting in the production of larger amounts of reactive oxygen species and hepatic oxidative stress (Fernandez et al. 2005).
murine peritoneal macrophages with TH also resulted in increased phagocytosis and chemotaxis (Forner et al. 1996, Ortega et al. 1996, 1999). Notably, a 10,000-fold greater concentration of TH did not affect macrophage function, suggesting that this effect is only present at physiological levels (Forner et al. 1996, Ortega et al. 1999). One study found an opposite effect on phagocytosis with macrophages from hypothryoid animals demonstrating higher phagocytosis and no effect of hyperthyroidism on phagocytic capacity (Rosa et al. 1995).

The generally observed pro-inflammatory effect of TH administration on macrophages suggests a shift towards an M1 phenotype. Incubation with T$_3$ indeed polarizes bone marrow-derived murine macrophages towards a pro-inflammatory M1 phenotype and inhibits M2 polarization (Perrotta et al. 2014). Polarization was accompanied by a change in TR$_{a_1}$:TR$_{b_1}$ ratio, suggesting that perhaps relative abundance of TR isoforms is associated with macrophage phenotype (Perrotta et al. 2014). It should be noted that these effects were achieved by incubating cells with supraphysiological levels of T$_3$ (500nM); therefore, these results are in contrast with the studies mentioned previously that find no effect of supraphysiological TH levels (Forner et al. 1996, Ortega et al. 1999). This could be explained by the degree of excess: 500nM is an approximately 250-fold higher concentration than circulating T$_3$ in euthyroid mice, whereas Forner and coworkers and Ortega and coworkers used a 10,000-fold higher concentration.

**Role of intracellular thyroid hormone metabolism in macrophage function**

Although the effects of extracellular thyroid hormone concentrations on macrophages are reasonably well characterized, the role of specific elements of intracellular TH metabolism in these effects has only recently been studied. The results indicate that adequate regulation of intracellular TH levels affects macrophage function via a combination of genomic and non-genomic pathways. The TH-induced increase in iNOS expression and activity, phagocytosis and bacterial killing is thought to be partly mediated via binding of TH to integrin $\alpha v \beta 3$ on the extracellular surface of the cell, which results in the rapid activation of the PI3K and ERK1/2 signalling pathways (Fig. 2A) (Chen et al. 2012).

Besides the extracellular binding of TH, regulation of intracellular TH levels was also recently shown to play an essential role in the pro-inflammatory response of macrophages (Kwakkel et al. 2014). D2, which converts T$_3$ to T$_4$, thereby regulating intracellular TH bioavailability, is induced in macrophages stimulated with bacterial endotoxin (lipopolysaccharide; LPS) together with TR$\alpha_1$ and MCT10, indicating a shift towards increased TH action during inflammation (Fig. 2A) (Kwakkel et al. 2014). Furthermore, D2 knockout resulted in impaired macrophage phagocytosis and blunted cytokine response to LPS stimulation (Kwakkel et al. 2014). These effects appear to be partly mediated via genomic pathways as knockout of TR$\alpha$, which is the predominant TR isofrom in macrophages, also results in aberrant macrophage function (Billon et al. 2014, Kwakkel et al. 2014). Macrophages from TR$\alpha$-knockout mice have impaired cholesterol efflux during atherosclerosis resulting in earlier plaque formation (Billon et al. 2014). Furthermore, macrophages that lack TR$\alpha$ demonstrate low-grade inflammation at baseline compared to controls, indicating an anti-inflammatory role for TR$\alpha$ (Billon et al. 2014). This suggests that attenuation of the rapid pro-inflammatory response generated by increased intracellular TH levels could be mediated via TR$\alpha$ (Fig. 2A).

**Thyroid hormone metabolism in tissue-resident macrophages**

Tissue-resident macrophages can vary widely in phenotype depending on which tissue they are from. TH metabolism has been specifically investigated in two well-characterized types of tissue-resident macrophages: Kupffer cells and microglia.

**Kupffer cells** As the resident macrophages of the liver, Kupffer cells are essential for liver homeostasis. This role is enhanced during infection and inflammation. Several studies by the same group have analysed the effect of TH administration on rat liver and the role of Kupffer cells in this process. T$_3$ administration induces oxidative stress in the liver (Tapia et al. 1997, 2006, 2010, Valencia et al. 2004, Fernandez et al. 2005, 2007a,b, 2008, 2009). This is thought to be mediated via Kupffer cells, which demonstrate hyperplasia, increased phagocytic capacity, increased ROS generation and tumour necrosis factor $\alpha$ (TNF$\alpha$) production in response to T$_3$ administration in vivo (Fig. 2B) (Tapia et al. 1997, Valencia et al. 2004, Fernandez et al. 2005, 2007b). Kupffer cell activation by T$_3$ triggers a cascade of responses in the liver including increased TNF$\alpha$ and interleukin-6 (IL-6) levels, which lead to the activation of liver STAT3 phosphorylation and nuclear factor kappa-B (NFkB) DNA binding. This ultimately increases liver iNOS expression and activity...
together with other markers of oxidative stress including decreased liver glutathione content (an important antioxidant) and higher protein oxidation (Fig. 2B) (Tapia et al. 1997, 2006, Valencia et al. 2004, Fernandez et al. 2005, 2007a,b). Conversely, another study found that T₃ inhibited STAT3 signalling in macrophages after LPS or IL-6 stimulation and had no effect on TNFα induction and NFκB activation in vitro (Contreras-Jurado et al. 2016). Both acute and chronic inflammation results in increased liver D₂ mRNA expression and activity (Kwakkel et al. 2014). This increase occurs independently of changes in serum TH levels and is thought to be caused by increased Kupffer cell activation resulting in higher D₂ activity. This suggests that Kupffer cells are also capable of local T₃ generation during inflammation, which could be an important mediator in the inflammatory response of these cells (Kwakkel et al. 2014).

The exact pathways through which the effects of T₃ are mediated in Kupffer cells are still unclear. It is possible that immediate non-genomic actions of TH are partly attenuated by late genomic TR effects that inhibit the initial pro-inflammatory response to TH in these cells.

Interestingly, the Kupffer cell-mediated induction of hepatic oxidative stress by T₃ appears to ameliorate the harmful effects of ischaemic reperfusion injury in the liver. Hepatic ischaemic reperfusion injury in rats results in severe liver damage (Fernandez et al. 2007a, 2008, 2009, Tapia et al. 2010). The effects of T₃ preconditioning were abolished by the addition of treatment with N-acetylcysteine, a powerful antioxidant, suggesting that the protective effect of T₃ on liver ischaemic reperfusion injury is achieved through

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**Figure 3**

Effects of thyroid hormone on microglia (reproduced with permission from Mori et al. 2015). Schematic representation of possible signal transduction pathways activated by T₃. Microglia contain various TH transporters (LAT2, OATP4a1 and MCT10) and TH receptors. Intracellular T₃ activates intracellular TRs (TRα1 and possibly TRβ1) and appears to also couple to various other factors, such as nitric oxide synthase (NOS), G-protein, PI3K and MAP/ERK. The receptors for gamma-aminobutyric acid (GABA) A and B are also involved in T₃-induced microglial migration but not in T₃-induced phagocytosis, the mechanism of which is unclear. The reverse mode of the Na⁺/Ca²⁺ exchanger (NCX) may be activated by Na⁺ influx by the Na⁺/K⁺ pump, resulting in Ca²⁺ influx, which in turn activates small-conductance Ca²⁺-dependent K⁺ channels (SK). This subsequently induces microglial migration and phagocytosis, possibly due to membrane hyperpolarization. Hypothetical signalling pathways for phagocytosis are indicated by the dotted line. Reproduced, with permission, from Mori Y, Tomonaga D, Kalashnikova A, Furuya F, Akimoto N, Ifuku M, Okuno Y, Beppu K, Fujita K, Katafuchi T, et al. (2015) Effects of 3,3’,5-triiodothyronine on microglial functions, Glia, volume 63, pages 906–920. Copyright (2015) Wiley Periodicals, Inc.
development of transient and reversible oxidative stress (Fernandez et al. 2008, 2009).

Microglia Microglia are the resident macrophages of the central nervous system and are derived from myeloid progenitor cells that migrate to the brain during foetal development (Mass et al. 2016). Due to the neurological phenotype associated with mutations in TH transporters, intracellular TH metabolism in brain cells has been assessed in detail. Both murine and human microglia express high levels of LAT2 in addition to MCT10 and OATP4a1, allowing them to transport TH into the cell (Wirth et al. 2009, Braun et al. 2011). In addition, microglia express both TRα1 and TRβ1 (Lima et al. 2001).

Physiological TH concentrations appear to be crucial for microglial growth and morphological differentiation (Lima et al. 2001). Hypothyroid rats showed delayed microglial growth and differentiation, whereas hyperthyroid animals displayed the opposite phenotype with accelerated microglial growth and differentiation (Lima et al. 2001). A recent study by Mori and coworkers assessed in detail the effects of T3 on microglial activation and the signalling pathways involved (Mori et al. 2015). T3 exposure increased migration, activation and phagocytosis in primary mouse microglia in vitro, indicating a shift towards a more mature and pro-inflammatory phenotype (Mori et al. 2015). These effects were found to be mediated via both genomic and non-genomic pathways (detailed in Fig. 3; reproduced with permission from Mori et al. 2015). Microglial migration and morphological changes associated with cell activation were dependent not only on TH transporters and TRs but also on gamma-aminobutyric acid (GABA)-A and GABA-B receptors, NOS, intracellular Ca2+ influx and G-protein-mediated signalling pathways including phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) (Mori et al. 2015). Conversely, the T3-induced stimulation of phagocytosis appeared to be partly mediated by other pathways that did not involve the GABA-A and GABA-B receptors (Mori et al. 2015).

Thyroid hormone metabolism in dendritic cells

Intracellular thyroid hormone metabolism in dendritic cells

DCs are known to express TRβ1 and to a lesser degree TRα1 (Mascanfroni et al. 2008). Furthermore, it was recently

![Figure 4](image-url)
reported that DCs are capable of active TH transport via transporters MCT10 and LAT2 and exhibit D2 and D3 enzymatic activity (Gigena et al. 2015 abstract 475, presented at the International Thyroid Congress).

Effects of extracellular thyroid hormone levels on dendritic cell function

TH stimulation has profound effects on DC phenotype. Incubation of human peripheral blood mononuclear cells with TH enhances their ability to differentiate into functional DCs (Mooij et al. 1994). Extensive work by the Pellizas group has shown that stimulation of murine bone marrow-derived DCs with physiological levels of T3 results in the initiation of the adaptive immune response by induction of DC maturation, increased interleukin-12 (IL-12) production, improved antigen cross presentation and enhanced DC ability to stimulate a cytotoxic T-cell response and trigger antigen-specific responses \textit{in vivo} (Fig. 4) (Mascanfroni et al. 2008, 2010, Alamino et al. 2015). Cell survival and ability to migrate to lymph nodes \textit{in vivo} are also enhanced (Alamino et al. 2015). The effects of T3 stimulation are mediated via the TRβ1 receptor and the Akt and NFκB pathways independently of the PI3K pathway (Fig. 4) (Mascanfroni et al. 2008, 2010). The promoter region of the TRβ1 gene contains an NFκB response element, which upregulates TRβ1 expression after T3 stimulation, suggesting a regulatory feedback loop (Mascanfroni et al. 2010). Dexamethasone potently inhibits and even reverses the effects of T3 in DCs (Montesinos et al. 2012).

The effect of T3 on DCs could be beneficial in anti-cancer vaccines. As DCs are antigen-presenting cells, a patient’s own DC can be loaded with tumour antigen \textit{ex vivo}, inducing DC maturation (Palucka & Banchereau 2013). The DCs are then re-administered to the patient resulting in a cytotoxic T-cell response against the tumour. Unfortunately, the effects of DC-based vaccines are frequently limited by the short lifespan of activated mature DCs and the risk of immune tolerance (Palucka & Banchereau 2013). This necessitates the use of costimulatory molecules that increase DC survival and immunogenicity. As T3 increases DC survival and DC ability to migrate to lymph nodes, T3 could potentially be of use in DC-based anti-cancer vaccines (Alamino et al. 2016). Indeed, vaccination with T3-stimulated DCs in a mouse melanoma model inhibited tumour growth and increased host survival (Alamino et al. 2015).

Summary

Neutrophils, macrophages and dendritic cells are cells of the innate immune system that are crucial for the host defence against invading pathogens. Thyroid hormone plays an important role in the function of these cells. Neutrophils, macrophages and dendritic cells have all been shown to contain essential elements required for intracellular TH metabolism and TH action, including TH transporters, deiodinases and TRs. Furthermore, circulating TH levels strongly affect innate immune cell function. In general, incubation with TH appears to have a pro-inflammatory effect in these cells. This is illustrated by the fact that ROS production and MPO activity are increased in hyperthyroid neutrophils (Videla et al. 1993, Fernandez & Videla 1995, Szabo et al. 1996, Magsino et al. 2000, Mezosi et al. 2005, Russo-Carbolante et al. 2005b, Marino et al. 2006). Higher TH levels also increase reactive nitrogen species production, phagocytosis and bacterial killing in macrophages (Forner et al. 1996, Ortega et al. 1996, Chen et al. 2012). In accordance with these effects, T3 has been shown to polarize macrophages towards a pro-inflammatory M1 phenotype, whilst inhibiting anti-inflammatory M2 markers (Perotta et al. 2014). In dendritic cells, TH administration also has pro-inflammatory effects, illustrated by increased cell maturation, pro-inflammatory cytokine production and the increased ability to elicit a cytotoxic T-cell response (Alamino et al. 2015).

Although the effects of circulating thyroid hormone levels on innate immune cells have been studied for decades, the mechanisms involved have only recently been assessed and are currently only partially understood. In all three cell types discussed, there appears to be an interplay of genomic and non-genomic pathways involved in the effects of TH on cellular function. In neutrophils, extracellular TH appears to induce its pro-inflammatory effects by binding to an unknown G-protein-coupled receptor whose downstream effects are mediated via the protein kinase C pathway (Fig. 1) (Mezosi et al. 2005). In macrophages, another non-genomic pathway has been described involving binding of extracellular TH to integrin αβ3, resulting in activation of the ERK and PI3K signalling pathways (Fig. 2) (Chen et al. 2012). The effects of T3 stimulation in dendritic cells are mediated via TRβ1 and the Akt and NFκB pathways (Fig. 4) (Mascanfroni et al. 2010). Besides the clear effects of altered extracellular TH levels, recent research has shown that several elements of intracellular TH metabolism
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appear to be essential for adequate pro-inflammatory neutrophil and macrophage function. In neutrophils, the thyroid hormone-inactivating enzyme D3 appears to play an important role during infection (Boelen et al. 2005, 2009, van der Spek et al. 2016). D3 induction results in decreased intracellular TH bioavailability and increased rT₃ levels, so the role of D3 during bacterial killing may appear contradictory given the effects of exogenous TH in these cells. However, D3 is a T₃-responsive gene that is known to be induced by T₃ in other tissues (Hernandez 2005). Whether a similar mechanism is present in neutrophils has not been studied to date, but this could potentially explain the similarity between the effects of higher extracellular TH levels and intracellular D3 activity. In macrophages, both D2 and TRα are required for correct cellular function, which is in accordance with the effects of extracellular TH as both these mechanisms result in increased intracellular TH action (Billon et al. 2014, Kwakkel et al. 2014). These studies convincingly demonstrate that adequate regulation of intracellular TH bioavailability is essential for neutrophil and macrophage function.

Conclusions

Circulating TH levels have a profound effect on neutrophil, macrophage and dendritic cell function. In general, increased TH levels result in an amplification of the pro-inflammatory response of these cells. Besides a pro-inflammatory effect of extracellular TH, the cellular response to pro-inflammatory stimuli appears to be dependent on functional intracellular TH metabolism, suggesting that TH metabolism plays an important role in host defence against infection. To date, this has best been demonstrated in macrophages.

Future research should focus on the intracellular pathways involved in the modulation of the immune response of innate immune cells by TH. Although a small number of recent promising studies have analysed the pathways that mediate the effects of TH stimulation in innate immune cells and the role of intracellular TH metabolism in innate immune cell function, still relatively little is known about the mechanisms involved. Whether the effects of increased TH levels and the functional role of intracellular TH metabolism in the immune response of these cells are in fact linked and part of the same mechanisms remains to be studied. Studies focussing on these issues would further elucidate the important connection between the endocrine and innate immune systems. This overview of the literature suggests that TH plays an important role in the host defence against infection through the modulation of innate immune cell function.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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