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Review

THP-1 cell line: An in vitro cell model for immune modulation approach

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ABSTRACT

THP-1 is a human leukemia monocytic cell line, which has been extensively used to study monocyte/macrophage functions, mechanisms, signaling pathways, and nutrient and drug transport. This cell line has become a common model to estimate modulation of monocyte and macrophage activities. This review attempts to summarize and discuss recent publications related to the THP-1 cell model. An overview on the biological similarities and dissimilarities between the THP-1 cell line and human peripheral blood mononuclear cell (PBMC) derived-monocytes and macrophages, as well as the advantages and disadvantages of the use of THP-1 cell line, is included. The review summarizes different published co-cultivation studies of THP-1 cells with other cell types, for instance, intestinal cells, adipocytes, T-lymphocytes, platelets, and vascular smooth muscle cells, which can be an option to study cell–cell interaction in vitro and can be an approach to better mimic in vivo conditions. Macrophage polarization is a relatively new topic which gains interest for which the THP-1 cell line also may be relevant. Besides that an overview of newly released commercial THP-1 engineered-reporter cells and THP-1 inflammasome test-cells is also given. Evaluation of recent papers leads to the conclusion that the THP-1 cell line has unique characteristics as a model to investigate/estimate immune-modulating effects of compounds in both activated and resting conditions of the cells. Although the THP-1 response can hint to potential responses that might occur ex vivo or in vivo, these should be, however, validated by in vivo studies to draw more definite conclusions.

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1. Introduction

Monocytes and macrophages belong to the innate immune compartment, in which their major roles are (i) recognition of foreign pathogens such as bacteria, fungi and viruses via interaction of their surface structures with different types of Pattern Recognition Receptors (PRRs), (ii) proliferation to increase the amount of cells that are able to eliminate pathogens, (iii) production of pro-inflammatory chemokines and cytokines, e.g. to recruit effector cells to the site of infection, and anti-inflammatory cytokines when the infection is under control and (iv) phagocytosis to engulf and digest pathogens [1]. Early studies indicated that THP-1 cells resemble primary monocytes and macrophages in morphological and functional properties, including differentiation markers [2–6].

2. Versatility of the THP-1 monocytic cell line

The THP-1 cell line is isolated from the peripheral blood of a 1-year old male patient suffering from acute monocytic leukemia [7]. This cell line has been widely used to study immune responses while cells are not only in the monocyte state but also in the macrophage-like state [8,9]. THP-1 macrophages, when stimulated with LPS, expressed MD2, CD14 and MyD88 genes, which are also required for LPS signaling in vivo. In addition to THP-1 cells, U937, ML-2, HL-60 and Mono Mac 6 cells have been also used in biomedical research. The basic difference between U937 and THP-1 cells is the origin and maturation stage. U937 cells are of tissue origin, thus at more mature stage, whereas THP-1 cells originate from a blood leukemia origin at less mature stage. Exposing U937 monocytes to 20 ng/ml phorbol-12-myristate-13-acetate (PMA) for 72 h [10] or to 0.1 μmol vitamin D3 for 24 h [11] induced the expression of the CD14 macrophage marker gene. Inflammatory marker genes (TLR-4, TNF-α, NF-κB and its subunit genes p52, p50, RelB, c-Real, p65) were expressed in U937 cells in a wide range of LPS stimulations of 0.3 ng/ml–2.5 μg/ml [12–14]. Sharif et al. [15] described that U937 monocytes responded to 2 μg/ml LPS with expression of cytokine, chemokine and stress genes at both 1 and 4 h of incubation, but at a very low level. This result was dramatically different from THP-1 monocytes and PBMC derived macrophages that were included in the same study. Comparison between THP-1 cells and PBMCs is addressed later in this review.

ML-2, a human myeloblastic sub-line from the ML-1 cell line, displayed morphological changes to a macrophage-like phenotype during cultivation in the presence of 5 ng/ml PMA for 48 h. This cell line was established in 1978, while the THP-1 cell line was developed in 1980, but the number of studies that used THP-1 as an in vitro cell model is much higher than for ML-2 [16]. The Mono Mac 6 human cell line was derived from peripheral blood of a patient with monoblastic leukemia. This cell line can be differentiated into a macrophage-like phenotype with the use of either PGE_2, PMA or LPS [17]. Mono Mac 6 is considered to be more suitable for the study of phenotypic and functional features of in vivo mature monocytes because of its ability to constitutively phagocytose antibody-coated erythrocytes [18] and mycobacteria [19,20]. In addition, it expresses the mature monocyte markers M42, LeuM3, 63D3, Mo2 and UCHMI, which could not be found on the THP-1 and U937 cell lines.

2.1. Differentiation of THP-1 macrophages

THP-1 cells in the monocyte state can be differentiated into a macrophage-like phenotype using either phorbol-12-myristate-13-acetate (PMA), 1α, 25-dihydroxyvitamin D3 (vD3) or macrophage colony-stimulating factor (M-CSF). Although the latter stimulus is biologically relevant in vivo, it is less frequently applied in vitro, unless in combination with IFN-γ. M-CSF is rather well-known to participate in the differentiation process of early stem cell-monoblasts into monocytes before they are released into peripheral blood [21,22]. Hence it plays a critical role in the in vitro macrophage differentiation lineage starting from bone marrow-derived monocytes. A, possibly naturally present, compound as vD3 is also known to induce the differentiation of THP-1 monocytes. THP-1 cell differentiation using 100 nM vD3 for 3 days resulted in macrophages less similar to PBMC monocyte-derived macrophages in terms of phagocytic activity and production of IL-1β and TNF-α than THP-1 macrophages generated using 200 nM PMA, also for 3 days [8,23]. Various macrophage phenotypic intermediates can develop from THP-1 cells during PMA stimulation at 2 days, such as adhering to culture plates, altering their morphology into flat and amoeboid in shape with well-developed Golgi apparatuses, rough endoplasmic reticula and large numbers of ribosomes in the cytoplasm [5]. Daigneault et al. [8] claimed that differentiation using 200 nM PMA for 3 days, followed by 5 days rest in culture media without PMA, increased the expression of macrophage markers. These included, for instance, cytoplasmic to nuclear ratio, mitochondrial and lysosomal numbers and differentiation-dependent cell surface markers, which developed in a similar pattern as do PBMC monocyte-derived macrophages.

Based on literature and on our experience [24–26], it can be summarized that PMA is the most effective differentiation agent to obtain mature THP-1 monocyte-derived macrophage with similarities to PBMC monocyte-derived macrophages. THP-1 monocytes can be fully differentiated into macrophages after at least 48 h of incubation at a minimal concentration of 100 ng/ml PMA (162 nM). It should take into account that too high PMA concentration can cause undesirable responses; particularly those that are NF-κB-derived, of the differentiated-macrophages. It is necessary to rest differentiated THP-1 macrophages at least 24 h in culture media without PMA in order to increase macrophage markers and decrease NF-κB gene clusters which have been up-regulated during differentiation. Cell adherence, high phagocytic capacity and expression of differentiation-dependent cell surface markers; CD-14, CD36, TLR-2, and CR3 (CD11b/CD18), can be used to assure fully differentiation of THP-1 macrophage.

2.2. Advantages and disadvantages of THP-1 cell line

Due to either financial or ethical constraints, inevitably connected to animal and human in vivo studies, and to the specific mechanistic insights that can be obtained, ex vivo or in vitro experiments become more relevant for the development of specific applications. Ex vivo systems have the advantage of their natural origin; however, donor viability and high individual variation can make analyses and interpretation of results more complex. Mouse or human cell lines, originating from cancerous cells, are important in vitro tools to study cellular functions, mechanisms and responses, as well as signaling pathways and nutrient and drug transport. These can support the eventual

in vivo proven pharmacological activity of drugs or health promoting efficacy of selected compounds for human consumption. Advantages of the use of THP-1 cell line over PBMC-derived monocytes or macrophages are listed as follows:

1. The average doubling time in THP-1 monocytes is around 35 to 50 h. Under growing conditions with the use of RPMI 1640 supplemented with 10% FBS, THP-1 cells can quadruple within three and a half days. This growing rate is much higher compared to that of PBMC-derived monocytes.
2. There is no reported evidence for the presence of infectious viruses or toxic products in THP-1 cells, making this cell line relatively easy and safe to use.
3. THP-1 is an immortalized cell line that can be cultured in vitro up to passage 25 (approx. 3 months) without changes of cell sensitivity and activity. PBMC-derived monocytes require inflammatory mediators, for instance, IL-1β, TNF-α or LPS, to function as survival factors to prevent apoptosis [27,28].
4. The availability of PBMC-derived monocytes is often limited and these cells cannot be stocked in liquid nitrogen. THP-1 cells can be stored for a number of years and by following an appropriate protocol, this cell line can be recovered without any obvious effects on monocyte–macrophage features and cell viability.
5. The homogeneous genetic background of THP-1 minimizes the degree of variability in the cell phenotype, which facilitates reproducibility of findings [29,30], whereas high variation from individual donors became a common problem for the application of PBMC-derived monocytes. This homogeneous background can be, at the same time, a disadvantage as effects may be genotype-dependent.
6. Isolation of leukocytes from buffy coat has to be performed within a week after the blood is withdrawn. Monocytes from buffy coat of healthy adults can vary from 2 to 10% of PBMC [31]. Contamination with other blood components (such as platelets) should be considered [29,30].

Unintended effects from in vitro differentiation of THP-1 macrophages, for instance, up-regulation of specific genes during the differentiation process might overwhelm mild effects of specific stimuli, particularly food-derived bioactive compounds [32] or can cause an increased sensitivity to LPS [33,34]. However, this might be less of an issue if strong stimuli, e.g. drugs or chemical compounds, are applied. A drawback of the use of cell lines is that the malignant background and the cultivation of cells under controlled conditions (outside their natural environment) might possibly result in different sensitivities and responses compared to normal somatic cells in their natural environment [35]. Also, possibly relevant interactions between the target cells and surrounding cells, as in natural tissues, cannot be easily mimicked. However, in vitro co-cultivation of THP-1 cells with neighboring cells might be an option to overcome this drawback.

2.3. Comparison cell functions/markers/responses between THP-1 cell line and human PBMCs

A number of publications have compared responses between the THP-1 monocytes and human PBMC–monocytes. These studies have included a variety of stimuli and findings as shown in Table 1. In most cases, both types showed relatively similar response patterns. However, differences have been reported in the degree of gene expression and cytokine secretion, as well as in gene expression baseline.

We have also performed a literature search to obtain data on a comparison of responses between THP-1 and human PBMC-derived macrophages. Reports on this, however, were fewer than those describing the monocytic phenotype. We summarized the remarks which have been made by the authors of these studies in Table 2.

A number of macrophage-markers, for instance, receptor expression, morphology and functional characteristics have been determined in differentiated THP-1 macrophages as shown in Table 3. However, it should be noted that different differentiating stimuli, like PMA or vD3, might lead to a different degree of differentiation. Also, basal expression of certain receptors or genes can be different.

Under certain conditions, THP-1 cells might less well resemble PBMC-derived monocytes or tissue macrophages (which are phenotypically and tissue-dependent; e.g. lungs, gut, liver and central nervous system) isolated from subjects with diseases, for instance, in obesity and chronic inflammation [49]. It might be possible, however, to mimic disease conditions by addition of regulating compounds into the THP-1 regular culture medium. For example, Miao et al. [50] mimicked diabetic conditions by culturing THP-1 monocytes in RPMI 1640 medium containing either 5.5 mM (normal) glucose, 25 mM (high) glucose or 19.5 mM mannitol for 72 h. The results indicated that diabetic marker genes, their coding and promoter regions were similarly expressed as those of blood monocytes from diabetic patients. Co-cultivation of THP-1 cells with other cell types, as described later in this review, can lead to a more relevant cell model to mimic the in vivo situation. For example, indirectly co-culturing THP-1 with the pre-adipocyte cell strain can mimic inflamed adipose tissue occurring under obese conditions [51]. These studies hint that THP-1 cells can potentially be an important model mimicking human blood cells, increasing relevance of their response for a clinical setting.

2.4. Polarization of THP-1 macrophages

Macrophages are plastic and heterogeneous cells, as a consequence of differentiation mechanisms that vary in responsiveness to stimuli and due to tissue localization. Distinct CD^{4+} T cell subsets, among other cell types, play major roles in regulation of the differentiation of macrophages into distinct phenotypes. IFN-γ induces M1, or classically activated, macrophages, while IL-4, IL-13 and IL-10 induce alternatively

Table 1
Examples of studies with compared responses from THP-1 monocytes vs human PBMC–monocytes.

Comparison	Stimulation condition	Findings	References
THP-1 vs PBMCs	LPS from *P. aeruginosa* or *E. coli* 10 ng/ml in time intervals up to 24 h	PBMC monocytes produced greater amount of inflammation-related cytokines, e.g. TNF-α, IL-6, IL-8 and IL-10 than do THP-1 monocytes	[35]
	LPS from *Salmonella minnesota* 100 ng/ml for 3 h	Up-regulation of TLRs 1, 2 and 4 in THP-1 monocytes. Up-regulation of TLRs 1–5 in PBMC monocytes	[36]
	Human oxLDL 50 μg/ml for 48 h	Baseline expression levels of [a]CNP, [b]NPR2, NPR3, TNF-α, IL-1β, IL-6, CD14 and CD68 were significantly higher in PBMC monocytes compared to THP-1 monocytes ($p < 0.05$)	[37]
	Polysaccharide from *Ganoderma tsugae* 4 and 40 μg/ml for 0.5 h	PBMC monocytes produced IL-1α and TNF-α in a similar fashion to THP-1 cell response	[38]
	Hyper-branched polysaccharide from *Ganoderma sinense* 0.0001–1000 μg/ml for 72 h	Cytotoxicity of the test compounds (XTT proliferation assay) was observed to be similar for THP-1 and PBMC–monocytes	[39]
THP-1 XBlue™ cells vs PBMCs	PHA 10 μg/ml for 48 h and 1 μg/ml LPS from *E. coli* for 72 h	Both cell types showed NF-κB activation with similar fold induction	[40]

[a] CNP: C-type natriuretic peptide, peptide with anti-inflammatory, anti-proliferative, and anti-migration properties.
[b] NPR: natriuretic peptide receptor.

Table 2
Examples of findings/remarks from THP-1 macrophage studies.

Stimulation condition	Findings/remarks	References
Parasite infection drugs; amphotericin B, miltefosine and sodium stibogluconate, for 1–4 days	*Leishmania amazonensis* infected-THP-1 macrophages exhibited great concordance to PBMC–macrophages with similar EC_{50} of the tested drugs	[41]
10–100 ng/ml LPS *E. coli* J5 for 6 h	THP-1 macrophages expressed the MD2, CD14 and MyD88 genes, which are also required for LPS signaling in vivo	[34]
2 μg/ml LPS for 1 and 4 h	THP-1 cell line provides an accurate and valid cell model system for evaluating the LPS response in macrophages	[15]
Others	THP-1 cells in both the monocyte and the macrophage state are useful tools for drug and compound-screening purposes	[42–45]
	THP-1 macrophages can be an alternative to replace PBMC–macrophages in terms of cytokine production, cell morphology and macrophage surface markers (0.1–10 μg/ml LPS)	[44]

activated or M2-macrophages [52–54]. The characterization is based on observations that M1-like macrophages play a role in producing pro-inflammatory cytokines and mediators, providing host defense against microorganisms and in tumor regression [55] by stimulating a Th1-driven immune response [56]. Expression of TNF-α, IL-1β, IL-6, IL-8 and IL-12 genes, and expression of PRRs, such as Toll like receptors (TLRs) and NOD-like receptors (NLRs), have been reported to be up-regulated during M1 activation [52,57]. Alternatively, M2 type macrophages participate in the resolution of parasite infection, tissue modeling, immune-regulation, allergy and tumor progression [58] by stimulating a Th2-driven immune response [59,60]. M2 macrophages have been further classified into three subsets: M2a induced by IL-4 or IL-13, M2b induced by exposure to immune complexes (IC)/TLR-agonists or IL-1-receptor and M2c induced by IL-10 [61]. These characteristics of M2 subtypes are hypothesized to be related to specific functions such as killing of pathogens, immune regulation and tissue remodeling as described by Mantovani et al. [61].

One of our THP-1 studies [25] described a protocol for THP-1 macrophage polarization. The PMA-differentiated macrophages (M0) were primed with fresh medium supplemented with 20 ng/ml IFN-γ + 1 μg/ml LPS for 6 h to differentiate into the M1 phenotype and with 20 ng/ml IL-4 for 24 h to the M2 phenotype. The results indicated that PMA-differentiated THP-1 macrophages were able to be polarized into the M1 or M2-like phenotypes utilizing the same stimuli as applied in vivo in mice and humans. Several known M1 and M2 marker genes were found to be up-regulated during THP-1 macrophage polarization. We concluded that TNF-α, IL-1β, IL-12p40, IL-6 and IL-8 can be used as M1 marker genes, while MRC-1, dectin-1 and DC-SIGN can be used as M2 marker genes. New M1 marker genes (LOX-1, CXCL1-3, CCL8, CCL19 and CSF3) and a new M2 marker gene (CCL13) have been identified in the THP-1 system, which should be further analyzed in polarized-PBMC-derived macrophages [25]. We have also performed a preliminary study, in which we tested polarization of THP-1 cells that were still in the monocyte state, applying the same M1- and M2-stimuli as mentioned above. Using monocytes as starting cell type, THP-1 cells did not change their morphology into macrophage-like, and only mild expression of some M1/M2 marker genes was observed (unpublished data). Spencer et al. [47] and Caras et al. [48] also reported the polarization of THP-1 macrophages (see Table 3 for stimulation conditions). The stimulation conditions of these two studies were a little different from Chanput et al. [25], likely resulting from different research aims. Spencer et al. [47] intended to understand interaction of adipocytes in insulin-resistant human subjects and the development of fibrosis that results from cytokine secretion by THP-1 macrophages. The result showed that THP-1 macrophages were polarized towards M2 macrophages after co-culturing with adipocytes and abundantly secreted TGF-β which activates the wound healing process. Caras et al. [48] aimed to study the effect of tumor-secreted soluble factors on macrophage polarization. THP-1 macrophages, incubated with supernatant from the primary tumor cells containing tumor-secreted soluble factors, switched towards a mixed M1/M2 phenotype. From all the above studies, it can be concluded that PMA-differentiated THP-1 macrophages appear to represent a simplified model to study macrophage polarization.

Table 3
Macrophage markers observed in differentiated THP-1 macrophages.

Macrophage markers	Stimulation condition	References
Increase of cytoplasm, mitochondria and ribosome numbers	200 nM PMA for 72 h	[8]
Expression macrophage receptors: CD11b, CD14 and CD36	10 nM PMA for 3 and 72 h	[9]
	5–100 ng/ml PMA for 48 h	[32]
	40 nM PMA for 48 h	[46]
Polarizing ability (plasticity)	M1	
	− 20 ng/ml LPS + 20 ng/ml IFN-γ for 24 h	[47]
	− 1 μg/ml LPS + 20 ng/ml IFN-γ for 6 h	[25]
	− 10 ng/ml LPS + 5 ng/ml IFN-γ for 72 h	[48]
	M2a	
	− 20 ng/ml IL-4 for 24 h	[25]
	− 25 ng/ml IL-4/25 ng/ml + IL-13 for 72 h	[48]
Macrophage morphology and adherence	160 nM PMA for 48 h	[5]
Macrophage function: phagocytosis, accumulate lipids and present antigens	10 nM PMA for 3 and 72 h	[9]
Resistance to apoptosis	200 nM PMA for 72 h	[8]

2.5. Co-cultivation of THP-1 cells with other cell types

Apart from studying responses of THP-1 cells in the monocyte or macrophage state as a single cell type, this cell model has been used in co-culture system with other cells, for instance, vascular smooth muscle cells, adipocytes, T-lymphocytes, platelets and intestinal cells.

2.5.1. Vascular smooth muscle cells

Co-cultures of THP-1 cells and human umbilical vein endothelial cells (HUVECs) [62] or human aortic endothelial cells (HAECs) [63] were used to study attraction of THP-1 monocytes to the atherosclerotic plague formation of endothelial cells in the inflammatory areas. The adhesion ability of fluorescently labeled THP-1 cells was determined [64–66]. Schildberger et al. [35] activated THP-1 and PBMC–monocytes before co-culturing with HUVEC and found that different cytokine profiles of HUVEC can be distinguished due to the amount of cytokine released from those two cell types. The observations led to the conclusion that THP-1 monocytes were chemically attracted by activated endothelial cells.

2.5.2. Adipocytes

Obesity-associated inflammation enhances macrophage infiltration in adipose tissue as a consequence of inflammatory cytokine production,

such as TNF-α and IL-6 [67]. Keuper et al. [68] established an in vitro model system for human adipose tissue by direct incubation of Simpson–Golabi–Behmel syndrome (SGBS) adipocytes (pre-adipocyte cell line) with THP-1 cells. It was found that close proximity of both cell types induced insulin resistance and apoptosis in SGBS cells. Spencer et al. [47] co-cultured primary human adipocytes with THP-1 macrophages using the transwell-method to examine whether their gene expression pattern was shifted towards a pro-inflammatory (M1) or an anti-inflammatory (M2) macrophage profile. The results indicated that co-culture with adipocytes induced a shift of THP-1 macrophages to the M2 phenotype. Because THP-1 cells and adipocytes did not come into contact with each other in the co-culture system, these findings suggested that adipocytes secrete soluble factors which promote polarization of macrophages.

2.5.3. T-lymphocytes

Jurkat T-cells are an immortalized T-lymphocyte cell line that is often used to study T cell signaling. Azenabor et al. [69] co-cultured *Chlamydia trachomatis* infected-THP-1 macrophages with Jurkat T-cells to study the induction of anti-inflammation in macrophages. It was reported that *C. trachomatis* mediated a switch from M1 to M2 macrophages due to high and low production of IL-10 and IFN-γ, respectively. Kanwar and Kanwar [70] used THP-1/Jurkat-T cell transwell co-culture as a model to study chronic gut inflammatory diseases, such as inflammatory bowel diseases (IBDs). The above studies show that the interaction between innate and adaptive immunity can be investigated via in vitro co-cultivation of THP-1 and T cells, which strengthens the capability of THP-1 polarization as previously discussed.

2.5.4. Platelets

Leukocyte–platelet interaction is important in the initiation and progression of atherosclerosis. Aslam et al. [71] incubated fluorescent dye-labeled human platelets with THP-1 cells. Risitano et al. [72] used co-culture of platelet-like particles with activated THP-1 monocytes to study mechanisms by which platelets mediate inflammation and immune pathways. These results led to the conclusion that this co-culture can be used as a model to mirror the interaction between platelets and monocytes.

2.5.5. Intestinal cells

Permeability, measured as transepithelial electrical resistance (TEER), of a Caco-2 cell layer (a human epithelial colorectal adenocarcinoma cell line), grown in transwells, could be decreased by co-culturing for 48 h with THP-1 macrophages, due to their secretion of TNF-α [73]. A similar finding was reported by Moyes et al. [74] that Caco-2 cells in a transwell system for 24 h with underlying THP-1 macrophages showed a lower TEER value, less organized epithelium and higher microparticle uptake. However, our preliminary findings showed results that contrasted to the aforementioned studies, as the TEER-value of the Caco-2 cell layer was not decreased by THP-1 macrophages after an incubation period up to 30 h (unpublished data). The differences of these studies were the number of seeded Caco-2 cells and the pore size of the transwell membrane. Moyes et al. [74] seeded 10^6 cells per transwell insert in a 6-well plate format (4.2 cm^2, 3.0 μm pore size), whereas we used 1.95×10^5 cells per transwell insert for 24-well plate format (0.6 cm^2, 0.4 μm pore size; corresponding to 1.36×10^6 cells for 6-well transwell format). Examples of co-cultivation studies of Caco-2 and THP-1 are by Cammarota et al. [75], Ishimoto et al. [76], Jiang et al. [77] and Girón-Calle et al. [78].

Hayashi et al. [79] found that THP-1 macrophages infected with either HSV-1 virus or viral components released chemokines, which attracted neutrophils. Transwell co-cultivation of THP-1 monocytes with A549 alveolar type II-like cell line suggested that cytokine gene expression of THP-1 monocytes could be regulated by chemokine secretion of respiratory epithelial cells [80]. Therefore, co-cultivation of THP-1 cells and specific tissue dependent-cells can be applied as a model to mimic in vivo chemotaxis. This conclusion is corroborated by the observation that THP-1 cell line expresses diverse cytokine and chemokine receptors [34] and is able to secrete chemokines to recruit other cells [15].

3. THP-1 cell model for immune modulation studies

3.1. THP-1 cells in LPS exposure studies

THP-1 cells have been widely used as an in vitro model of human monocytes and macrophages in mechanistic studies of inflammatory diseases [2–4,6]. It has long been believed that inflammatory monocytes are recruited and differentiated into macrophages at the site of infection [81]. In in vitro experiments, these cells are often described as being in an activation state [52,82–84], which can be obtained by stimulating THP-1 cells with inflammatory activators, for instance, LPS or pro-inflammatory cytokines for 3–6 h [15,26,85]. Chanput et al. [86] and Sharif et al. [15] showed that THP-1 cells exposed to LPS responded with a change in expression of a number of inflammation-related genes (IL-1β, IL-6, IL-8, IL-10 and TNF-α). These responses can already be detected within 1 h of incubation and some genes maintained their expression even after 6 h of incubation. In addition, exposure of THP-1 monocytes to LPS results in activation of the NF-κB transcription factor, which orchestrates a gene expression program leading to the activation of inflammation, cell proliferation, differentiation, migration and cell survival, which are mediated through the release of chemokines and cytokines [15,86].

Monocytes are blood circulating cells, whereas macrophages can be found only at the site of infection/inflammation (so called inflammatory-monocyte derived macrophages) or at the tissue/lymph nodes (so called tissue-resident macrophages) [87]. Due to the circulation of monocytes throughout the body, these cells can easily access the site of infection and respond fast in recognition of invaders or pathogen derived-substances. It has been shown that monocytes were recruited to the skin after injection of fluorescent latex beads, and subsequently ingest these. Most of the recruited monocytes remained at the injected area and differentiated into macrophages. In the meantime, some of the latex-bead-carrying monocytes did migrate from the skin to the lymph nodes where they differentiated into DCs [88,89]. The results shown in Chanput et al. [86] demonstrated that THP-1 monocytes responded faster to LPS than do PMA-differentiated THP-1 macrophages, which are defined as inflammatory-monocyte derived macrophages. THP-1 monocytes maximally expressed IL-1β, IL-6, IL-8, IL-10 and TNF-α genes after 3 h of LPS stimulation, while gene expression of THP-1 macrophages appeared to be maximal after 6 h. Although the fold induction in gene expression was relatively similar, secretion of cytokines, phagocytic activity and release of prostaglandin E_2 (PGE_2) were higher in THP-1 macrophages than in monocytes [13,86]. This result is in line with the finding of Takashiba et al. [33] and Schwende et al. [9], which can be interpreted as spontaneous responses of monocytes towards invasion of foreign substances and as effective clearance of invaders and infected cells by phagocytosis and oxidative burst of macrophages. Simultaneous exposure of THP-1 cells to LPS and food compounds, coupled with gene expression analysis has been proposed as a useful in vitro screening tool to select inflammation modulating-food compounds [86].

3.2. THP-1 in drug exposure studies

Inflammation in the liver can be caused by infections from viral hepatitis, bacteria, protozoa, as well as by toxins and drugs. Inflammatory reactions are induced by the activation of liver resident macrophages, so called Kupffer cells, and infiltrating monocytes and neutrophils at the injured liver tissue. The activation of these cell types causes the release of large amounts of pro-inflammatory cytokines and chemokines [90]. It was suggested that THP-1 cells were useful to examine inflammatory

responses mediated by drugs [91]. A number of research groups have used the THP-1 cell line to determine the release of pro-inflammatory cytokines and chemokines upon treatments with hepatotoxic drugs such as mebendazole [91], ximelagatran [92], terbinafine [93] and troglitazone [94]. The study of Edling et al. [92,94] used parent drug compounds to treat THP-1 cells, and later investigated drug metabolite formation. These authors concluded that parent drug compounds can be metabolized by THP-1 cells since concentrations of parent drugs were proportional to those for drug metabolites. Although, THP-1 cells might not be suitable to test parent drug compounds since drugs are mostly metabolized in the liver by hepatocytes, but they can be suitable to study inflammatory responses provoked by drugs, that cause immune-mediated liver injury [95]. Plattner et al. [96] used PMA-differentiated THP-1 macrophages to investigate lectin-mediated drug delivery. Among several tested lectins, wheat germ agglutinin (WGA) exhibited the highest binding capacity as determined by fluorescence intensity as well as high phagocytic activity and cytokine production. WGA-functionalized THP-1 macrophages were able to take up 55% of nanoparticles, suggesting an approach for treatment of infectious disease provoked by parasites, bacteria and viruses.

3.3. THP-1 in natural product exposure studies

A variety of natural compounds have been tested in the THP-1 cell model in either the resting or inflammation-activated state. Examples of such studies are listed in Table 4.

4. Commercially available THP-1 engineered cell lines

THP-1 cells are human monocytic cells that are known to constitutively express most TLRs. In recent years, InvivoGen has engineered THP-1 cell lines as a tool to facilitate answering specific research questions. Examples of engineered THP-1 cell lines, as well as published articles using them as tools, are listed below.

4.1. THP1-XBlue™ cells–NF-κB/AP-1 SEAP reporter monocytes

4.1.1. THP1-XBlue™

THP1-XBlue™ is derived from the THP-1 cell line via transfection with a reporter construct expressing a secreted embryonic alkaline phosphatase (SEAP) gene under the control of a promoter that is inducible by the transcription factor NF-κB and AP-1. If such transcription factors are activated via PRRs, such as TLR 2–5 and 8–9, THP1-XBlue™ cells will induce the expression of NF-κB and AP-1, and subsequently these secrete SEAP. The cell response can be monitored using QUANTI-Blue™, a medium that turns purple/blue in the presence of SEAP. This readout allows researchers to facilitate monitoring of TLR-induced NF-κB/AP-1 activation.

4.1.2. THP1-XBlue™–CD-14 and the THP1-XBlue™–MD2–CD14

The THP1-XBlue™–CD-14 and the THP1-XBlue™–MD2–CD14 cell lines have been designed to study the role of the CD-14 gene, and of MD2 in cooperation with the CD14 gene, respectively. THP1-XBlue™–defMyD cells are THP1-XBlue™ cells deficient in MyD88 activity. They are unable to respond to the activity of receptors whose signaling is dependent on MyD88, such as most TLRs and IL-1Rs, but they remain responsive to MyD88-independent receptors, such as NOD1 and TNFR. The readout is also QUANTI-blue™ linked to other responsive transcription factors.

4.1.3. THP1-Blue™ NF-κB cells

THP1-Blue™ NF-κB cells are specifically designed for monitoring the NF-κB signal transduction pathway in a physiologically relevant cell line. They are highly responsive to PRR agonists that trigger the NF-κB pathway even when low concentrations are applied.

4.1.4. THP1-Blue™ ISG cells

THP1-Blue™ ISG cells are derived from THP-1 cell line by stable integration of an interferon regulatory factor (IRF)-inducible SEAP reporter construct. THP1-Blue™ ISG cells secrete SEAP under the control of an ISG54 promoter in conjunction with IFN-stimulated elements

Table 4
Examples of natural product exposure studies using THP-1 cell model.

	Stimulating condition	Findings	Reference
THP-1 monocytes	Besifloxacin (antibacterial agent) at 0.1–30 mg/L on 10 μg/ml LPS-stimulated cells for 18 h	Inhibition of pro-inflammatory cytokine production	[97]
	A concentration range of aloe vera gel + 200 ng/ml LPS for 24 h	Suppression of LPS induced TNF-α production	[98]
	1 μg/ml LPS +/− TNF-α (0.1–100 ng/ml) for 48 h	Autocrine and exocrine regulation of IL-10 production	[99]
	1 μg/ml LPS +/− anti-TNF-α (0.1–100 ng/ml) for 48 h		
	1–25 μM monascus-fermented metabolites for 24 h on ovalbumin induced inflammation	Suppression of COX-2 and iNOS gene expression, and production of NO and PGE2	[100]
THP-1 macrophages	0.2–1 mg/ml Daesiho (Korean herbal mixture) + 100 ng/ml LPS for 24 h	Suppression of TNF-α, IL-1β and IL-6	[101]
	30 min nobiletin (citrus flavonoid) stimulation followed by 30 nM PMA incubation for 24 h	Suppression of scavenger receptor gene expression; SR-A, SR-PSOX, CD36, and CD68	[102]
	50–200 μg/ml *Astragalus* polysaccharide + 1 μg/ml LPS for 24 h	Inhibition of TNF-α and IL-1β production and MAPK/NF-κB pathway	[103]
	5–15 μg/ml ethanolic extracts of Brazilian red propolis for 24 h	Promotion of [a]ABCA1 expression and cholesterol efflux	[104]
	50 μM curcumin for 0–12 h	Induction of apoptosis through JNK/ERK pathways	[105]
	6–25 μM 25-hydroxycholesterol-3-sulfate for 24 h followed by 100 ng/ml LPS incubation for 3 h	Reduction of inflammatory response via PPARγ pathway	[106]
	LPS-stimulation followed by β-glucans incubation from mushroom	Reduction of pro-inflammatory genes; IL-1β, TNF-α and COX-2	[26,85]
	10–100 μg/ml β-glucans from *Candida albicans* for 1–6 h of incubation	Increase expression of TNF-α and IL-8 genes and H_2O_2 release	[107]
	100 μM palmitate for up to 27 h	Induction of pro-inflammatory gene expression; TNF-α, IL-8 and IL-1β	[108]
	20 μg/ml pulmonary surfactant protein A for 24 h +0.1–10 ng/ml LPS for another 24 h	Expression of TNF-α gene Additive effects on TNF-α gene expression	[109]
	400 ng/ml Shiga toxin-1 for 0–72 h +/− 200 ng/ml LPS	Activation of TNF-α and IL-1β genes and secretion of TNF-α and IL-1β	[110]
	400 ng/ml Shiga toxin-1 for 2–6 h +/− 200 ng/ml LPS	Activation of MIP-1α, MIP-1β, GRO-β, and IL-8	[111]

[a] ABCA1 (ATP-binding cassette transporter A1), a membrane transporter contributing to HDL biogenesis.

resulting in high responsiveness to PRR agonists that trigger the IFN signaling pathway, such as LPS and double stranded nucleic acid.

Needham et al. [112] performed a TLR signaling assay using different engineered cell lines including THP1-XBlue™ with combinatorial structural diversification of lipid A in LPS from *Escherichia coli* to generate a combinatorial library of lipid A molecules. The results showed activation of several TLRs, as well as secretion of pro-inflammatory cytokines, in a structure-dependent manner indicating the sensitivity of THP1-XBlue™ in response to LPS with different lipid A structures. Grodzki et al. [113] used macrophage-derived PMA-differentiated THP1-XBlue™ cells to investigate whether oxygen tension influences THP-1 cell differentiation and primary macrophage functions. The readout was examined using SEAP expression in differentiated THP1-XBlue ™ cells cultured in 18% versus 5% O_2 in the absence and presence of LPS. Decreasing the oxygen tension to 5% O_2 significantly increased the rate of PMA-induced macrophage differentiation and secretion of cytokine after LPS stimulation. It decreased, however, phagocytic activity. Gostner et al. [114] used THP1-Blue™ NF-κB cells to investigate the effect of the malaria parasite on NF-κB signaling.

Although the THP-1 engineered-cells can be very helpful for screening purposes due to their straight forward response, broader effects on cell functions and metabolisms might not be covered. There is no report published yet that compares normal THP-1 cells and engineered-reporter THP-1 cells with respect to possibly altered signaling pathways and other cellular parameters that may have been affected by the engineering process.

4.2. THP1 inflammasome test cells

The inflammasome is a multi-protein complex which promotes the maturation of inflammatory cytokine genes, such as IL-1β and IL-18. A number of inflammasomes have been described, such as NLRP1, NLRP3, NLRC4, ASC and pro-caspase-1. The NLRP3 inflammasome is the most extensively studied and also the most versatile due to its broad response to a wide range of PAMPs and non-microbial danger-associated molecular patterns (DAMPs). The inflammasomes NLRP3, ASC and pro-caspase-1 are activated by translocation of a transcription factor which consequently initiates a reaction cascade that results in the activation of pro-inflammatory cytokines such as IL-1β and/or IL-18. Three subsets of the THP1 inflammasome test cells, for instance, THP1–defASC cells (knock-down of the expression of ASC), THP1–defNLRP3 cells (knock-down of the expression of NLRP3) and THP1-null cells (fully competent for NLRP3 and ASC activities), have been released in 2012. Rossol et al. [115] showed that both THP1–defASC cells and THP1–defNLRP3 cells were much less susceptible to a danger signal from extracellular Ca^{2+}, compared to the normal THP-1 cell line due to the role of these two knocked-down inflammasomes which are responsible for the signal. The other study used THP1–defNLRP3 and THP1 null to study the importance of NLRP3 inflammasome on stimulation by unfolded proteins [116]. More information on these two studies can be found on the InvivoGen website http://www.invivogen.com/inflammasome-test-cells.

5. Conclusion and suggestion

Health of humans and animals is strongly related to a proper balance of immune functions. These can be mediated by many factors, including diets: so called “immune modulation”. To study such a topic, the use of in vitro cell line can be meaningful because it enables to minimize the effects of culture period and genetic variation, the restraints posed by ethical issues, donor accessibility and availability, and provides mechanistic information. Cells in vivo always work in cross talk with other cells and tissues as a network. Co-culture systems in in vitro or ex vivo conditions may present a step forward to mimic the in vivo situation. Examples of this are THP-1 macrophages with adipocytes, intestinal cells, dendritic cells or T cells.

The THP-1 cell line is known to be a suitable model to represent a simplified, suitable and reliable model to study monocyte and macrophage functions/responses, macrophage differentiation and possible effects from external stimuli in the surrounding environment. This suggests the option to use such cells for compound screening purposes, product development and quality controls, for instance, when raw materials and production processes are changed and altered bioactivities of target compounds are expected. However, an investigation on how well the findings obtained from in vitro can be correlated to those from ex vivo or in vivo should be firstly considered. Future human interventions will be required to achieve definite evidence of the bioactivity of food and non-food compounds and their support in maintenance of human health.

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