Chapter 14
THP-1 and U937 Cells

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Abstract Monocytes are circulatory precursor cells from myeloid origin that can develop into macrophages or dendritic cells upon migration from the blood stream to tissues. Both macrophages and dendritic cells are professional antigen-presenting cells. Monocytes and their macrophage and dendritic-cell progeny serve three main functions in the immune system. These are phagocytosis, antigen presentation, and cytokine production. THP-1 and U937 are (pro-) monocytic cell lines that can, also in vitro, be differentiated into either various types of macrophages or into dendritic cells.

This chapter describes how to grow THP-1, resp. U937 cells, how to differentiate these into more specialised phenotypes such as various macrophage types or dendritic cells, how to read-out their responses to stimuli and it gives examples of how such cell lines have been used into studying the effects of food compounds.

Keywords THP-1 • U937 • Cell line • Monocyte • Macrophage

14.1 Origin and Some Features of THP-1 and U937 Cells

The THP-1 cell line is a human monocytic leukaemia cell line which was established in 1980 by Tsuchiya et al. (1980). It was derived from the blood of a patient with acute monocytic leukaemia. THP-1 cells resemble primary monocytes and macrophages in morphology and differentiation properties. THP-1 cells show a large, round single-cell morphology and express distinct monocytic markers. Nearly all THP-1 cells start to adhere to culture plates and differentiate into macrophages after exposure to phorbol-12-myristate-13-acetate (PMA, also known as TPA,12-O-tetradecanoylphorbol-13-acetate; see below for details).

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THP-1 cells have some technical advantages over human primary monocytes or macrophages. For instance, their genetic background is homogeneous which minimizes the degree of variability in the cell phenotype. Another technical advantage is that genetic modification of THP-1 cells by small interfering RNAs (siRNAs), in order to down regulate the expression of specific proteins, is relatively simple (Chanput et al. 2014). Furthermore, monocyte-derived macrophages can be polarized into M1, M2a, M2b, and M2c cells. Spencer et al. (2010) published a protocol for THP-1 cell differentiation, showing that THP-1 cells represent a simplified model to study monocyte-macrophage polarization (Qin 2012).

U937 is a pro-monocytic, human myeloid leukaemia cell line and was isolated from the histiocytic lymphoma of a 37 year old male (Sundstrom and Nilsson 1976). This cell line exhibits many characteristics of monocytes and is easy to use. A virtually unlimited number of cells can be prepared and they are relatively uniform. This cell line has been an important tool in the investigation of the mechanisms involved in monocyte-endothelium attachment (Liu et al. 2004). These leukaemia cells have been used as the experimental model to elucidate mechanisms of monocyte and macrophage differentiation. A genetic analysis by Strefford et al. (2001) showed that U937 bears the t(10;11)(p13;q14) translocation. This results in a fusion between the MLLT10 (myeloid/lymphoid or mixed-lineage leukaemia) gene and the Ap-3-like clathrin assembly protein PICALM (Clathrin assembly lymphoid myeloid leukaemia), which is likely important for the tumorous nature of the cell line (Strefford et al. 2001).

14.2 Stability, Consistency and Reproducibility of the System

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. As far as our information reaches, U937 is used also at higher passage numbers (see e.g. Strefford et al. 2001). THP-1 as well as U937 cells can be stored for a number of years and, provided an appropriate protocol is followed, the cell lines can be recovered without any obvious effects on monocyte-macrophage features and cell viability (Chanput et al. 2014 and references therein).

14.3 Relevance to Human In Vivo Situation

Cell lines always have a malignant background, which presents a significant risk of experimental bias. The cultivation of cells under controlled conditions and outside their natural environment possibly results in different sensitivity and responses compared to normal somatic cells in their natural environment (Schildberger et al. 2013).

Also, possibly relevant interactions between the target cells and surrounding cells, as in natural tissues, cannot be easily mimicked. In vitro co-cultivation of THP-1 or U937 cells with neighbouring cells might be an option to make this drawback less pronounced (Chanput et al. 2014).

14.4 Other Models with the Same Applicability

Next to THP-1 and U937 cells, ML-2, HL-60 and Mono Mac 6 cells are used in biomedical research. U937 cells are the most frequently used. 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 leukaemia origin at less mature stage (Chanput et al. 2014). Because Mono Mac 6 is able to phagocytose antibody-coated erythrocytes (Ziegler-Heitbrock et al. 1988) and mycobacteria (Friedland et al. 1993; Shattock et al. 1994) it is thought more suitable for the study of phenotypic and functional features of in vivo mature monocytes. Also, it expresses mature monocyte markers that cannot be found on the THP-1 and U937 cell lines, such as M42, LeuM3, 63D3, Mo2 and UCHMI. As THP-1 and U937 are very frequently used, we focus here on these two cell lines.

14.5 General Protocol of Culturing THP-1 Cells

Roswell Park Memorial Institute (RPMI) 1640 medium is a commonly used medium for THP-1 as well as for U937 cells. Alternatively, DMEM (Dulbecco's Modified Eagle's Medium; Morton 1970), also supplemented with 10 % FBS, is used to grow U937 cells. RPMI 1640 medium was originally developed by Moore et al. (1967), at Roswell Park Memorial Institute to culture human leukemic cells in suspension and as a monolayer. The formulation is based on RPMI-1630 medium and uses a bicarbonate buffering system and has alterations in the amounts of amino acids and vitamins. When RPMI is properly supplemented, it has a wide applicability for supporting growth of many types of cell cultures including HeLa, Jurkat, MCF-7, PC12, PBMC, astrocytes and carcinomas.

In most of the studies, the RPMI medium is supplemented with a combination of foetal bovine serum (FBS) and antibiotics. FBS is also known as foetal calf serum (FCS) and is obtained from whole blood by removing blood cells, platelets and fibrinogen. Serum includes all proteins not involved in blood clotting and all electrolytes, antibodies, antigens, hormones and exogenous substances. Foetal bovine serum is obtained via collection at a slaughterhouse. In some studies, foetal bovine serum is heat-inactivated in order to destroy heat-labile complement proteins (Biowest).

In many studies, the antibiotics penicillin and streptomycin are used to supplement the medium in order to prevent bacterial contamination of cell cultures due to their effective combined action against gram-positive and gram-negative bacteria. Penicillin, originally purified from the fungus *Penicillium*, acts directly and indirectly by interfering with the turnover of the bacterial cell wall and by triggering the release of enzymes that further alter the cell wall respectively. Streptomycin was originally purified from *Streptomyces griseus* and acts by binding to the 30S subunit of the bacterial ribosome, which leads to inhibition of protein synthesis and death in susceptible bacteria (Waksman 1953).

In some studies, cells were also supplemented with HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid). HEPES is a zwitterionic organic chemical buffer and is widely used in many biochemical reactions and as a buffering agent in some cell culture media. These buffers have pK_a values between 6.0 and 8.0, high solubility, limited effect on biochemical reactions, membrane permeability, are chemically and enzymatically stable and easy to prepare.

14.6 Differentiation of THP-1 and U937 Monocytes into Macrophages

As already mentioned before, nearly all THP-1 cells start to adhere to culture plates and differentiate into macrophages after exposure to PMA. Also U937 pro-monocytes differentiate into mature monocytes or into macrophages upon PMA-treatment. Typical exposure to PMA is for 48 h (Zhang et al. 2010; Gillies et al. 2012; Moreno-Navarrete et al. 2009; Cam and de Mejia 2012). The temperature and atmosphere, if described, was the same for each study, namely 37 °C and 5 % CO_2 respectively. Different stimuli were used, depending on the aim of the study.

Subsequently, macrophages can be further differentiated into subsets. Typical markers for M1-type macrophages are transcription or production of TNF-α, IL-1β, IL12-p40, IL-6, IL-8 and LOX-1, and for M2-type macrophages MRC-1, dectin-1, and DC-SIGN (Chanput et al. 2010).

Based on literature and on our experience, 0.5×10^6 THP-1 monocytes fully differentiate into macrophages after 48 h incubation at a minimal concentration of 100 ng/ml PMA (162 nM), followed by washing twice with culture media without PMA and a resting period of 24 h (Chanput et al. 2014), resulting in macrophages with a high phagocytic capacity for latex beads and expressing cytokine profiles that resembled PBMC monocyte-derived macrophages after exposure to TLR ligands (Chanput et al. 2014).

Human promonocytic leukaemia U937 cells differentiate into monocytes and macrophages by use of various agents such as retinoic acids, 1,25-dihydroxyvitamin D3 (VD3; at 100 nM, i.e. 42 ng/ml, Rots et al. 1999), and 12-O-tetradecanoylphorbol-13-acetate (TPA; at 20 ng/ml, ca. 32 nM) (Chun et al. 2001).

14.7 Differentiation of THP-1 and U937 Monocytes into Dendritic Cells

THP-1 monocytes are described to differentiate into mature dendritic cells by transferring them to serum-free medium, and subsequently treating them with a mixture of IL-4, GM-CSF, TNF-α and ionomycin. These hematopoietic cell line-derived DCs are highly pure and monotypic, and display the morphologic, phenotypic, molecular, and functional properties of DCs generated from human donor-derived monocytes or CD34+ hematopoietic progenitor cells (see also Chap. 17). During differentiation into mature DCs, the cells exhibit de novo cell-surface expression of CD83, CD80, CD86, CD40, CD206, CD209, CD120a, CD120b, and intracellular synthesis of IL-10, increase their endocytotic capacity, and acquire the characteristic stellate morphology (Berges et al. 2005). In the THP-1 monocytes, mRNAs of tight junction molecules, occludin, tricellulin, JAM-A, ZO-1, ZO-2 and claudin-4, -7, -8, and -9 were detected by RT-PCR. In mature DCs that had elongated dendrites, mRNA and protein of JAM-A were significantly increased compared to the monocytes (Ogasawara et al. 2009).

Exposure of U937 to a self-peptide from apolipoprotein E, Ep1.B, induces DC-like morphology and surface marker expression in U937 (Stephens et al. 2008).

14.8 Controls to Test Viability and Performance of the Model

The by far most commonly used and imperative control test for analyses that are based on any kind of THP-1 or U937 derived cell is the possibility of cytotoxic effects of the test samples. For such checks, various methods and kits are in common use in various laboratories. Examples are assessment of functional mitochondrial reductase, with tetrazolium salts such as MTT, XTT and lamar blue. Alternatively, cell membrane integrity, degree of cell lysis via measurement of lactate dehydrogenase and apoptosis kits are used.

14.9 Critical Notes

Regularly, solvents such as ethanol or DMSO are used to facilitate dissolution of test compounds. Also, sometimes a mimic of digestion is applied to the samples prior to analysis. It is highly recommended to determine the toxicity of such solvents or digestion reagents, as well as their effect on the read-out parameters to be used for the cell assay, in a titration that at the least covers the eventual concentration to be used in the assays, and also for a relevant range of higher and lower concentrations (at least a factor 10 lower and higher than eventually applied conditions). Similar recommendations are valid for the use of buffers, salt concentrations and pH-ranges etc.

14.10 Read-Out of the System

Most commonly used read-out systems for responses of THP-1 or U937 cells are transcription or production of relevant cytokines. Which cytokines are relevant depends on specific experimental design. Gene expression of IL-1β, IL-6, IL-8, IL-10, TNF-α, iNOS, COX-2 and NF-κB is measured in many studies. This is done by RT-PCR, Western blot and ELISA. Examples that have been used to read out the effects of various food compounds on resp. THP-1 and U937 cells are presented in Tables 14.1 and 14.2.

Transcription can be measured by various PCR-methods, mostly and preferably quantitative PCR such as qPCR or RT-qPCR. Advantages of using PCR are its ease, speed and sensitivity, and possibly its low cost. A clear disadvantage is that not mRNAs are physiologically relevant, but the eventual gene product i.e. the cytokine.

Cytokines are most commonly measured by ELISA-methods or by FACS, for which a variety of commercial kits are available. An important advantage is relevance as protein products are quantified, and the standardized kits that are on the market that facilitate comparison of read-outs between various laboratories. An important drawback associated to such commercial kits is their cost.

THP-1 cells can also be used to study expression and activation of nuclear receptors. Gillies et al. (2012) studied the effect of omega-3-PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and found that EPA-rich oil activated human peroxisome proliferator-activated receptor α (PPAR α) and PPAR β/α with minimal effects on PPARγ, liver X receptor, retinoid X receptor, farnesoid X receptor, and retinoid acid receptor γ (RARγ).

Notably, several studies looked at the intracellular production of reactive oxygen species (ROS) by THP-1 cells. During inflammation, ROS production is induced by inflammatory cells in order to kill pathogens. However, ROS also act on inflammatory cells themselves, thereby altering the intracellular redox balance and functioning as signalling molecules involved in the regulation of inflammatory and immunomodulatory genes. ROS plays a key role in the control of transcription factors, like NF-κB and activator protein-1 (AP-1), which are involved in the gene expression of both inflammatory and immune mediators. ROS can either activate or inactivate these transcription factors by chemically modifying critical amino acid residues within these proteins or on residues of accessory proteins of the respective signalling pathways (Jabaut and Ckless 2012).

ROS production can be measured by the intracellular ROS assay. In the study of Wu et al. (2011), 2′,7′-dichlorofluorescein (DCF) is used to measure ROS production. The cell-permeant 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) (also known as dichlorofluorescein diacetate) is a chemically reduced form of fluorescein used as an indicator for ROS in cells. Upon cleavage of the acetate groups by intracellular esterases and oxidation, the non-fluorescent H_2DCFDA is converted to the highly fluorescent 2′,7′-dichlorofluorescein (DCF). The chemically reduced and acetylated form of 2′,7′-dichlorofluorescein (DCF) is non-fluorescent until the acetate groups are removed. The fluorescence can be measured by a flow cytometry, fluorimeter, microplate reader, or fluorescence microscope.

Table 14.1 Food components tested on THP-1 cells

Compound	Analysed response	Method	Result	References
Lunasin	Levels of nitrite PGE2 TNF-α iNOS, COX-2, p-p65, and p-Akt	Griess reaction PGE2 EIA monoclonal assay ELISA Western blot	Reduced COX-2, iNOS, NO, PGE2, TNF-α levels Inhibited activation of p-AKT and NF-κB p65 Reduced αVβ3 intensity	Cam and de Mejia (2012)
Melittin	Serum lipids, pro-inflammatory cytokines TNF-α, NF-κB and IL-1β expression	Serum lipid analysis, ELISA (TNFα, IL8-β), protein analysis, Western blot	Decreased total cholesterol and triglyceride levels, high HDL-C Decreased TNF-α, IL-1β and NF-κB	Kim et al. (2011)
Lactoferrin	NF-κB Lactoferrin IL-6, MCP-1, IL-8, and cyclophilin A	Western blot ELISA RT-PCR	Lactoferrin negatively related to inflammatory markers Decreased IL-6, MCP-1, IL-8 and NF-κB	Moreno-Navarrete et al. (2009)
Casein α s1 (CSN1S1)	CSN1S1	Western blot, Real-time PCR, ELISA, immunocytochemistry, immunofluorescence	Human CSN1S1 may possess an immunomodulatory role beyond its nutritional function in milk	Vordenbaumen et al. (2011)
β-glucans	NO production Hydrogen peroxide (H_2O_2) IL-1β, IL-8, NF-κB and IL-10	Griess reagent Horseradish peroxidise mediated oxidation of phenol red RT-qPCR	No H_2O_2 or NO production Decreased induction of IL-1β, IL-8, NF-κB and IL-10	Chanput et al. (2012)
Barley glucan, quercetin and citrus pectin	Expression and secretion of IL-1β, IL-6, IL-8, IL-10, TNF-α, iNOS, COX-2, NF-κB, AP-1 and SP-1	RT-PCR (gene expression) Cytometric bead array (CBA) analysis (cytokine secretion)	Induced inflammation-related cytokines, COX-2 and NF-κB genes	Chanput et al. (2010)
Saturated fatty acids (SFAs)	NF-κB, TNF-α, IL-8, ROS production	Luciferase assay, Western blot, ELISA flow cytometer and microscopy, LAL endotoxin assay	Increased ROS production, activation of PRR signaling pathways	Huang et al. (2012)

(continued)

Table 14.1 (continued)

Compound	Analysed response	Method	Result	References
Omega-3-PUFAs, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)	Expression and activation of nuclear receptors	RNA extraction, reverse transcription, and real-time PCR, microarray, Human PPARα, PPAR β/δ, PPARγ, LXRβ, RXRα, and FXR reporter assay system	EPA-rich oil activated human peroxisome proliferator-activated receptor α(PPAR α) and PPAR β/α with minimal effects on PPARγ, liver X receptor, retinoid X receptor, farnesoid X receptor, and retinoid acid receptor γ(RARγ)	Gillies et al. (2012)
Proanthocyanidin	IL-6 and TNF-α	ELISA, cytotoxic assay	Decreased IL-6 and TNF-α	Tatsuno et al. (2012)
Curcumin	Expression of lipid genes	Quantitative RT-PCR	Increased expression of the fatty acids transporter CD36/FAT and the fatty acids binding protein 4 FABP4 Increased FOXO3a activity	Zingg et al. (2012)
Caffeic acid (CA), ferulic acid, m-coumaric acid, and chlorogenic acid	ROS TNF-α secretion	DCF-D ELISA RT-PCR, comet assay, cell migration assay	CA induces ROS, IL-1β and TNF-α production CA exhibits pro-oxidative and pro-glycative effects during the glycation process	Wu et al. (2011)
Cyanidin 3-O-beta-glucoside (C3G)	mRNA expression, Iκβα, NF-κB P65, TNF-α, IL-6 secretion	Real-time PCR ELISA, western blot	Increased mRNA expression and secretion of TNF-α and IL-6 Inhibition of Iκβα phosphorylation, thereby inhibiting NF-κB activity	Zhang et al. (2010)
Flavonoids (catechin, EGCG, luteolin, quercetin, rutin)	TNF-α, IL-1β intracellular ROS production NF-κB activity	ELISA Fluorescence RT-PCR, western blotting	All flavonoids inhibited TNF-α, IL-1β, and COX-2 Inhibits intracellular ROS production Inhibition of NF-κB activity	Wu et al. (2009)
Quercetin and catechin (flavonoids), S100B (RAGE ligand)	Gene expression of TNF-α, IL-1β, MCP-1, IP-10, PECAM-1, β2-integrin and COX-2 ROS production	RT-PCR, Western blot, cytokine ELISA assay, intracellular ROS assay	S100B (RAGE ligand) increased gene expression of TNF-α, IL-1β, MCP-1, IP-10, PECAM-1, beta2-integrin and COX-2 Quercetin and catechin inhibited these gene expressions, eliminate ROS	Huang et al. (2006)

Table 14.2 Tested food components on U937 cells

Compound	Analysed response	Method	Result	References
Salmon calcitonin (sCT) Hyaluronic acid (HA)	mRNA expression of NR4A1, NR4A3, and MMP1, -3 and -13	RT-PCR	Both sCT and HA attenuated activated mRNA expression of NR4A1, NR4A2, NR4A3, and MMP1, -3 and -13	Ryan et al. (2013)
Soluble toll-like receptor 2 (sTLR2)	Immunodepletion of sTLR2 IL-8 levels Cell viability	Western blot ELISA Duoset MTT assay	Increased IL-8 production	Henrick et al. (2012)
Quercetin	Expression of inflammatory genes, phosphorylation of JNK and c-Jun, IκBα degradation	PCR, immunoblotting	Attenuated expression of TNF-α, IL-6, IL-8, IL-1β, IFN-γ IP-10, COX-2 and PTP-1B Attenuated phosphorylation of c-Jun N-terminal kinase (JNK), c-Jun, decreased serine residue 307 phosphorylation IRS-1 Attenuated IκBα degradation in MΦs Suppression of insulin-stimulated glucose uptake	Overman et al. (2011)
Human casein α s1 (CSN1S1)	CSN1S1 mRNA and protein amount GM-CSF mRNA	ELISA	Detection of CSN1S1 mRNA and protein Upregulation of GM-CSF mRNA	Vordenbaumen et al. (2011)
Xanthones	Expression of TNFα, IL-6, IP-10, PPARγ, MAPK, AP-1, NF-κB	Quantitative PCR, immunoblotting	α- and γ-MG attenuated expression of TNFα, IL-6, IP-10	Bumrungpert et al. (2010)
Billberry juice (quercetin, epicatechin, and resveratrol)	Lipid peroxidation Levels of CRP, IL-6, IL-15, TNF-α and MIG NF-κB activation	D-ROMs test Sandwich immunoassay, fluorescence HPLC	Decreased CRP, IL-6, IL-15 and MIG (all target genes of NF-κB, also TNF-α) Increased TNF-α Inhibition of NF-κB activation	Karlsen et al. (2010)
Grape powder extract (GPE)	MAPKs, NF-κB AP-1, TNF-α, IL-6, IL-1β, IL-8, IP-10, COX-2, insulin resistance c-Jun, JNK, p38, EIk-1 Iκβα	PCR, immunoblotting, BioPlex suspension array system from BioRad	Induction of TNF-α, IL-6, IL-1β, IL-8, IP-10, COX-2 Attenuated activation of MAPKs, NF-κB, AP-1 Decreased phosphorylation of c-Jun, JNK, p38, EIk-1 Degradation of Ikβα	Overman et al. (2010)

(continued)

Table 14.2 (continued)

Compound	Analysed response	Method	Result	References
Grifola frondosa	NFκB (HT-29 cells) TNF-α, MCP-1 ROS (HT-29 cells)	NF-κB reporter gene dual-luciferase assay ELISA Fluorescence	Suppressed TNF-α expression	Lee et al. (2010)
Carvatrol	COX-2 expression	Transcription assays	Suppressed COX-2 expression	Hotta et al. (2010)
Cysteine	IL-1β levels Precursor IL-1β	ELISA Western blot	Increase in secreted pro-IL-1β and IL-1β levels	Iyer et al. (2009)
Nutrient mixture (NM) Quercetin, naringenin Hespzeretin Tea catechins Lysine, proline, arginine and N-acetylcysteine	COX enzymatic activity COX protein expression Specific mRNA levels NF-κB activation	Western blot analysis RT-PCR Phosphorylated p65 immunoassay	Inhibition of COX-2 enzyme activity Downregulation of COX-2 and pro-inflammatory cytokine protein expression levels by reduced NF-κB activation	Ivanov et al. (2008)
N-acetylcysteine, d-alpha-tocopherol acetate and ascorbic acid (antioxidants) Caffeic acid (CA)	NF-κB binding activity Intracellular peroxides content	Gel shift assay Formation of a fluorescent derivative of DCF	CA inhibits NF-κB binding activity and protein tyrosine kinase activity N-acetylcysteine, d-α-tocopherol acetate and ascorbic acid did not inhibit apoptosis, but affect NF-κB activity	Nardini et al. (2001)

Many studies using U937 have been used to analyse properties of poly-phenolic compounds. Responses like inflammatory gene expression and NF-κB activity are commonly measured. The effect of quercetin on the phosphorylation of JNK and c-Jun and the degradation of IκBα was measured by Overman et al. (2010). The c-Jun N-terminal kinases (JNKs) are members of a group of serine/threonine (Ser/Thr) protein kinases from the mitogen-activated protein kinase (MAPK) family. The JNKs act within a protein kinase cascade and are activated by dual phosphorylation by MAPK kinases, but can also be activated by pro-inflammatory cytokines including TNF-α and IL-1β. Moreover, the JNK pathway is activated in the innate immune response following the activation of various members of the Toll-like receptor family by invading pathogens. The JNK pathways appears to act as a critical intermediate in signalling in the immune system. There is increasing evidence that JNK is activated following sensing of internal stress events, such as protein misfolding. Studies also showed that bacterial, fungal, prion, parasitic, or viral infections activate JNK which may influence important cellular consequences such as alterations in gene expression, cell death, viral replication, persistent infection or progeny release, or altered cellular proliferation. The exact mechanism of JNK activation under each of these circumstances remains not fully clear, but there may be involvement of Toll-like receptors, direct pathway modulation through interaction with upstream protein regulators or the activation following an ER stress response (Bogoyevitch and Kobe 2006).

Overman et al. (2011) found that quercetin attenuated the phosphorylation of JNK and c-Jun, which means a decrease in JNK activation. They also observed attenuation in degradation of IκBα. Overman et al. (2010) showed the same results for grape powder extract. Degradation of IκB is a seminal step in activation of NF-κB. The IκB kinases (IKKs) lie downstream of the NF-κB-inducing kinase (NIK) and activate NF-κB by phosphorylation of IκBα. This leads to IκBα degradation and release of NF-κB. NF-κB is a key regulator of the pro-inflammatory cytokine release, so an inflammatory response is induced. In U937 monocytic cells, IL-1β and TNF-α induced IκB-dependent transcription equally (Nasuhara et al. 1999). These results are contradicting with the results from studies with quercetin on THP-1 cells, in which quercetin showed anti-inflammatory effects. It is possible that these cell lines respond differently to quercetin, simply because the cell lines are not the same.

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