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The human leukemia cell line, THP-1: A multifacetted model for the study of monocyte-macrophage differentiation

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Summary. THP-1 is a human monocytic leukemia cell line. After treatment with phorbol esters, THP-1 cells differentiate into macrophage-like cells which mimic native monocyte-derived macrophages in several respects. Compared to other human myeloid cell lines, such as HL-60, U937, KG-1, or HEL cell lines, differentiated THP-1 cells behave more like native monocyte-derived macrophages. Because of these characteristics, the THP-1 cell line provides a valuable model for studying the mechanisms involved in macrophage differentiation, and for exploring the regulation of macrophage-specific genes as they relate to physiological functions displayed by these cells.
Key words. Atherosclerosis; cellular differentiation; gene expression; foam cells; lipoproteins; phorbol esters; transcription factors.

The mononuclear-phagocyte system

The 'mononuclear-phagocyte system' consists of tissue macrophages and their precursor cells, monocytes[105]. These cells are considered to be a 'system' because of their common origin, their similar morphology, and their common functions, including rapid phagocytosis. This 'system' is dynamic and is represented in almost all tis-

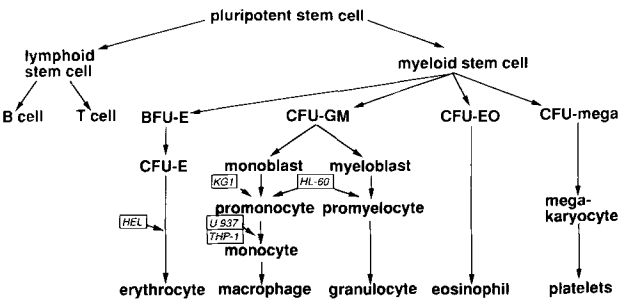

Figure 1. Hematopoietic cell differentiation; outline of the differentiation of hematopoietic stem cells. Some of the more frequently used myeloid leukemia cell lines are boxed. The arrow indicates their differentiation potential.

sues, where it has the potential to exert a modulatory role in tissue homeostasis and in immunological and inflammatory responses. Some of the tissues where macrophages are prevalent include: liver (Kupffer cells), lung (interstitial and alveolar macrophages), serous cavities (pleural and peritoneal macrophages), bone (osteoclasts), brain (microglial cells), placenta, intestinal wall, connective tissue, spleen, lymph node, bone marrow, breast milk[46].

Monocytes and macrophages are all derived from hematopoietic stem cells (fig. 1). Similarly to other cells of hematologic origin, monocytic precursors undergo an orderly differentiation process into monocytes. Pluripotent stem cells committed to myeloid development form the colony-forming unit for granulocytes-monocytes (CFU-GM), and leave the proliferative pool[96]. These cells differentiate into monoblasts, which evolve into promonocytes, the first morphologically identifiable cells in the series[106]. The transit time in the bone marrow from the first monocytic precursor to the mature monocyte is approximately 6 days. In the absence of localized inflammatory foci, monocytes migrate in a random way into the different tissues, where they terminally differentiate into macrophages, with morphological and sometimes functional characteristics typical for the tissue. The multifunctional nature of the monocyte implies that this cell has to undergo a very complex differentiation process, involving finely tuned changes in gene expression. This terminal tissue-specific differentiation is the result of tissue-specific stimuli, and represents a unique feature of the macrophage[46]. Macrophages have a life span in the range of months.

The process of blood cell formation and differentiation, by which a small number of self-renewing stem cells give rise to lineage-committed progenitor cells that subsequently proliferate and differentiate to produce the various kinds of circulating blood cells, is sustained through a family of glycoprotein hormonal growth factors[68,96]. Members of this group include various CSFs, interleukins, interferons, and erythropoietin. Other biological compounds whose implications are more strictly limited to macrophage differentiation (and activation) include the active vitamin D compound, 1,25 $(OH)_2D_3$, γ-IFN, and TNF (reviewed in Collins[29]). It needs, however, to be stressed that all the above-mentioned mediators are interacting in a complex network. How the final effect of such an interacting network of mediators on the cell is obtained via different or interacting receptor mechanisms and intracellular signal transduction pathways[80] is unclear at this moment, and awaits further research.

The initial concept that limited the function of the monocyte/macrophage to that of a scavenger cell in search of debris and unwanted material is an underestimate of this cell's powerful role in normal physiology and pathology[69]. Phagocytes respond in a unique way to external stimuli, and therefore they occupy a pivotal role in infectious processes, the modulation of immunological responses, and inflammation in its widest interpretation[102]. Firstly, they can interact with extracellular molecules, internalize them, and submit them to metabolic changes. These molecules may be free in solution or may form part of the structure of invading organisms. Secondly, macrophages secrete products, ranging in molecular mass from 32 (superoxide anion) to 440,000 (fibronectin), showing biological activities ranging from induction of cell growth to provocation of cell death[78]. So many secretory products have been demonstrated for very few other cells, and it enables the macrophage to intervene in several physiological processes, especially inflammatory reactions. This secretion process is largely dependent on the metabolic state of the macrophage, which in its turn is dependent upon its interaction with the environment. Thirdly, macrophages interact with other cells of the immune system such as lymphocytes, to which they present antigen, an important step in the generation of a humoral immune response. Finally, they possess receptors for lymphokines, the regulatory proteins released by lymphocytes; upon interaction with these cytokines, macrophages become 'activated', and subsequently carry out their tumoricidal and microbicidal activity even more effectively[1]. The 3 most important cytokines involved in this activation process include γ-IFN, granulocyte-macrophage colony stimulating factor (GM-CSF) and TNF[46]. TNF is actually produced by macrophages exposed to endotoxin and therefore opens the possibility for auto-activation. The most important functions of the mononuclear phagocyte system are summarized in table 1.

THP-1 cells as a model

Studies of monocytes/macrophages are often impeded by the limited amounts of human monocytes available. Fortunately, several lines of human leukemia cells, which are blocked at certain steps of the differentiation process (fig. 1), and which can be induced to differentiate into macrophages by several stimuli, are available[29,51]. These cell lines allow the investigation of relatively homogenous groups of cells during different stages of mat-

Table 1. Functions of human mononuclear phagocytes

Microbicidal activity
Tumoricidal activity
Chemotaxis
Phagocytosis
Pinocytosis
Antigen presentation
Secretion: – reactive oxygen species
– polypeptide hormones
– sterol hormones
– complement components
– coagulation factors and other enzymes
– extracellular matrix proteins
– binding proteins
– bioactive lipids
Respiratory burst
Modulatory function in several tissues

For references see Johnston[96] and Nathan[78].

uration and differentiation. All these cell lines can be induced to differentiate into macrophage-like cells by the phorbol ester, PMA. This review will focus on the monocytic leukemia cell line, THP-1, which displays commitment towards macrophage differentiation. This cell line was isolated by Tsuchiya et al.[100] from a boy suffering from acute monocytic leukemia. It resembles the human monocyte with respect to numerous criteria such as morphology, secretory products, oncogene expression, expression of membrane antigens, and expression of genes involved in lipid metabolism. In contrast to native human monocytes, a cell line such as THP-1 offers the additional advantage of a homogeneous population, which markedly facilitates further biochemical study. Under the influence of the phorbol ester, PMA, THP-1 cells stop proliferating and differentiate into macrophage-like cells[12]. The differentiation of the THP-1 cell is associated with a dramatic alteration in cell morphology. After the initiation of this differentiation process, cells acquire a variety of shapes, their nucleus becomes more irregular, and many phagocytic vacuoles can be recognized in their cytoplasm. This differentiation process is also associated with an increased adherence to tissue culture plastic. Furthermore, differentiated THP-1 cells display a decrease in forward scatter characteristics upon flow cytometry, which indicates that shortly after differentiation cell volume decreases[12].

It is evident that PMA is an artificial stimulator of monocyte-macrophage differentiation. Preliminary attempts to identify a more natural inducer of differentiation in THP-1 cells have been only partly successful. The naturally occurring active vitamin D compound, 1,25 $(OH)_2D_3$, induces only a partial differentiation of THP-1 cells[76] (Auwerx et al., unpublished data). This last substance, however, uses a completely different signal transduction pathway from PMA (steroid receptor versus protein kinase C). None of the cytokines and growth factors we have tested (including IL-1, IL-2, γ-IFN, TNF, GM-CSF) have the capacity to induce differentiation by themselves. It is, however, quite conceivable that a combination of these agonists may be required to mimic the full spectrum of activity induced by PMA. Recently, Lubbert and Koeffler[61] did indeed report that a combination of γ-IFN and TNF could inhibit colony formation of THP-1 cells synergistically.

Changes in expression of proto-oncogenes

Proto-oncogenes are important cellular regulatory molecules. They are implicated in signal transduction and thus they can have wide ranging effects on basic cellular functions such as the control of proliferation or differentiation[16]. In view of the association of abnormal oncogene structure and expression with hematologic malignancies, myeloid cell lines provide a good model system for the study of oncogene expression during cell proliferation and differentiation. Differentiation of monocytes or myeloid cell lines into macrophages has been associated with altered expression of various oncogenes[29]. A summary of these changes in oncogene expression is provided in table 2. On the one hand the expression of some oncogenes (such as c-myc or c-myb) has been reported to decrease, while on the other hand the expression of other oncogenes (such as c-fos) increases during macrophage differentiation. Finally there are some oncogenes, such as N-ras, whose expression does not change upon differentiation. In contrast to the available information for the HL-60 cell line (reviewed in refs 29 and 61) relatively little is known about oncogenes in the THP-1 cell line.

Since PMA (or TPA) is often used to induce differentiation in myeloid cell lines, we studied the expression of two proto-oncogenes, known to interact with the 'TPA responsive element (TRE)', i.e. c-fos and c-jun. Although the fos protein is a transcriptional activator it cannot bind to DNA unless it forms a complex with a transcription factor from the jun/AP-1 family[27, 84, 91]. The fos and jun oncoproteins bind as a heterodimeric transcription complex to the TRE, a unique cis-acting regulatory sequence[4, 17]. The association between fos and jun is mediated via a distinct domain in both proteins, characterized by the occurrence of interspaced leucine residues, and called 'leucine zipper'[53, 58, 91]. Although previous reports have shown that in fibroblasts PMA induces both fos and jun mRNA[57, 88], it is only very recently that data became available for hematopoietic cells[11, 59, 94]. Our

Table 2. Change in oncogene expression during human macrophage differentiation

Increased expression	Unchanged expression	Decreased expression
c-fms[90, 95]	jun D*	c-myb[40]
c-fos[19, 40, 71, 74]	N-ras[90]	c-myc[40, 42, 85, 90, 111]
c-jun[11, 59, 94]		
c-mos[56]		
c-src[38]		
jun B*		

*de Groot et al., unpublished data.

data indicate that in THP-1 cells, as in fibroblasts, c-fos and c-jun were coinduced[11]. This coinduction of c-fos and c-jun in THP-1 cells was most striking in the monocytic THP-1[11] and KG-1 cells (Auwerx et al., unpublished data). In HL-60 cells and in HEL cells, induction of these 2 oncogenes was not as tightly linked, since PMA induced mainly fos mRNA while it had only a limited effect on c-jun mRNA. A similar uncoupling of c-fos and c-jun induction was detected in THP-1 cells after stimulation of the adenylate cyclase pathway[11]. Since the observed changes in c-fos and c-jun mRNA levels are reflected in changes in the protein levels and hence in alterations in the formation of transcription complexes binding to a TRE, the uncoupling of the induction of these two oncoproteins might have important effects on gene regulation. Indeed, both c-fos and c-jun are required for optimal activation of responsive genes. The uncoupling of c-fos and c-jun could limit the formation of fos/jun heterodimeric transcription complexes in favor of other combinatorial complexes. The change from coupled to uncoupled induction might furthermore be involved in the generation of an increased diversity of cellular responses to a limited number of transducing signals.

In contrast to this transient induction of the c-fos and c-jun genes upon PMA-induced differentiation, c-myc gene expression has been reported not to change, while the c-myb gene was reported to be down-regulated during the differentiation process in THP-1 cells[59]. The activity of a differentiation-associated tyrosine-kinase p93-c-fes was also elevated in differentiating cells of the myeloid lineage, including THP-1 cells[97]. On the contrary, the expression of the c-fes gene was much less abundant in myeloid cells resistant to differentiation. Interestingly, cells resistant to myeloid differentiation, such as K562 cells, became capable of differentiating after transfection of the c-fes gene[113].

Although the alterations in prevalence of oncoproteins could change the various signal transduction pathways in these cells dramatically, it is not yet clear whether they act as potential master-switches, and translate the short-term effects of extracellular stimuli (such as the differentiation inducers PMA or 1,25 $(OH)_2D_3$) into long-term responses such as differentiation[24, 70, 75]. Further study is, however, still needed to clarify whether the observed changes in the activity of these oncogenes is only an associated phenomenon or is causally linked to the differentiation process, and to evaluate whether changes in oncogene expression in native monocyte-derived macrophages are similar to those in the myeloid leukemia lines investigated.

Changes in membrane antigen expression

Differentiation of myeloid precursors into mature phagocytic cells is associated with the acquisition of phagocytic and microbicidal capacity, two main functions involved in host defense. Membrane molecules play an important role in triggering these phagocytic and microbicidal reactions[3], and again, THP-1 cells provide a good model for studying alterations of membrane antigens (table 3). CD4 is one of these membrane glycoproteins. Current models propose that CD4 is an adhesion molecule with an affinity for class II histocompatibility antigens[15]. It is also the receptor for HIV[62]. Other surface receptors important for the recognition of foreign antigens and subsequent triggering of host defense are the complement receptors and the FcRs. Three types of leukocyte FcR (FcRI to FcRIII) have been identified by functional criteria, by distinct biochemical properties, and by their reactivity with monoclonal antibodies[3, 48, 103]. All the FcRs belong to the immunoglobulin gene superfamily. FcRI binds human IgG with high avidity while FcRII and FcRIII bind with much lower affinity.

Table 3. Membrane antigens and receptors reported on the human THP-1 cell line

– CD4[12, 32]	– TNF receptor*
– CD30[44]	– C3b receptor[12, 100]
– Factor-X-receptor (Mac I)[2]	– LFA-1[45]
– Factor-Xa-receptor[2]	– Fibronectin receptor[45, 104]
– FcRI[12]	– Leu M1[37]
– FcRII[12]	– Leu M2[37]
– GM-CSF receptor[23]	– Leu M3[37]
– HDL receptor*	– HLA-DR antigens[6, 82]
– LDL receptor[7, 8, 43]	– Scavenger receptors[43, 49, 50, 109]

* Auwerx et al., unpublished data. Abbreviations used: GM-CSF: granulocyte-macrophage colony stimulating factor; FcR: IgG Fc receptor; HDL: high density lipoprotein; LDL: low density lipoprotein; LFA: lymphocyte function associated antigen; C3b: complement 3b.

We ourselves studied the changes in membrane antigen expression during the differentiation of the THP-1 cells. The density of the complement C3b receptor increases during PMA-induced differentiation. Marked changes in the expression of the different FcRs were also detected during PMA-induced THP-1 differentiation. The mean fluorescence intensity on staining with an anti-FcRI and anti-FcRII mAb decreased, while FcRIII remained absent. The decrease in FcRI expression could be explained by a change in steady state level of its mRNA, while FcRII mRNA levels did not change, pointing to post-transcriptional regulation of FcRII expression. The undetectable FcRIII expression correlates with the absence of FcRIII mRNA in THP-1 cells.

Several lines of evidence supported the hypothesis that the changes in FcR and C3b receptor were linked to growth arrest and cell differentiation. First, the change in expression only occurred at concentrations of PMA able to block cell proliferation and induce differentiation. Second, the changes in FcR and C3b receptor expression developed gradually over a period of 48 h, in parallel with the time-course of the differentiation process and coinciding with the increased adherence to tissue culture dishes and the change in FSC and morphology of the THP-1 cells. This slow decrease in FcRI and FcRII expression contrasted sharply with the quick disappearance

of CD4 expression which occurred almost immediately after treatment of the cells with PMA. Third, these changes in FcR expression could be reproduced by 1,25 $(OH)_2D_3$, another agent capable of inducing macrophage differentiation[61], which does not use the PKC pathway. Fourth, short-lived stimulation of PKC with diacylglycerol did not result in the described changes in the FcR or the C3b receptor. Finally, inhibition of PMA-induced PKC activation by the addition of the PKC inhibitor H-7 did not inhibit the effect of PMA on FcR or C3b receptor expression. In addition to these differentiation-linked changes in membrane antigen expression, there is also ample evidence for altered expression of these proteins under the influence of cytokines. Indeed, Faltynek et al. showed that in THP-1 cells cell surface levels of CD4 decrease upon treatment with γ-IFN[32], while Arend et al. demonstrated that the expression of γ-IFN-induced FcR could be inhibited by LPS and IL-1 in THP-1 cells[15].

Several of the changes in the expression of membrane antigens observed in differentiating THP-1 cells have also been noticed during differentiation of the monocyte to the macrophage[28, 35, 60, 83]. A diminished expression of CD4 and an increase in prevalence of C3b are typical for macrophage differentiation of peripheral blood monocytes. In this context, a decrease of FcRII (but not FcRI) has been reported during differentiation of peripheral blood monocytes[28]. No reports of FcRI expression on native macrophages are yet available. It is therefore hard to estimate the potential importance of this finding in THP-1 cells. It is, however, unlikely that FcRI has an important role in vivo, because a complete deficiency of this receptor was shown not to compromise immune defenses in vivo[25]. The absence of FcRIII on differentiated THP-1 cells contrasts with the reported induction of FcRIII on cultured monocytes[28] and with the presence of these receptors on mature tissue macrophages[14]. The reason for this lack of FcRIII can be explained in several ways. First, it is possible that although THP-1 cells can undergo some differentiation, they can never attain a completely differentiated state necessary for FcRIII expression. The late appearance of FcRIII on differentiating monocytes might indeed indicate that the capacity of FcRIII expression only occurs in a very differentiated cell. Alternatively, it is conceivable that THP-1 cells have lost the capacity to make FcRIII. Selective loss of gene expression in cell lines is not uncommon. Despite this, the THP-1 cell once again appears to be a valuable model for the study of another important aspect of macrophage biology, i.e. membrane receptors.

Secretory proteins

One of the reactions of macrophages to physiological stimuli is the secretion of a wide variety of physiologically active substances ranging from polypeptide hormones and enzymes to reactive oxygen species. The THP-1 cell model can also be employed to study the secretory function of the macrophage. Several proteins secreted by the mature macrophage have been reported to be secreted by THP-1 cells (reviewed in refs 46 and 78; see table 4).

Table 4. Proteins reported to be secreted by the human monocytic THP-1 cell line*

Polypeptide hormones	*Enzymes*
TNF-a[6, 73]	Lipoprotein lipase[9, 99]
IL-1 a and IL-1b*	Lysozyme[100]
CSF-1 or M-CSF[36]	
Erythrocyte differentiation factor[77]	*Binding proteins*
PDGF-1 and 2[89]	Apolipoprotein E[9, 99]
Thymosin B4[41]	
Killer T cell activating factor[63]	
Monocyte chemotactic factor[66]	

*See text for references.
Abbreviations: TNF: Tumor necrosis factor; IL: Interleukin; M-CSF: Macrophage colony stimulating factor; EDF: Erythrocyte differentiation factor; PDGF: Platelet-derived growth factor.

The THP-1 cell has been used extensively to study the production of cytokines and peptide hormones. Most information exists on interleukin-1 (IL-1) production and its regulation by various stimuli in THP-1 cells[33, 34, 52, 54, 55, 65, 73, 82, 92, 101]. Two forms of IL-1 have been cloned in mouse and man, IL-1α and IL-1β (reviewed in ref. 31). Each gene consists of seven exons. The two IL-1's are initially synthesized as 31 kDa precursors and share only small stretches of amino acid homology (26% for the human form). Although the two forms are structurally distinct, they share the same properties and bind to the same receptor[39]. THP-1 cells have been shown to possess an enzymatic activity, typical of monocytes, which converts IL-1β to its active form[52]. Both LPS and PMA induce IL-1β mRNA levels in THP-1 cells[34, 101]. The induction of IL-1β with LPS is transient, which contrasts with the more stable induction seen with PMA[34]. Also TNF, lymphotoxin, GM-CSF and 5' azacytidine have been reported to induce IL-1β transcription[33, 54, 82]. Opposite to the inductive effects seen by the above-mentioned agents, it has been reported that heat shock reduces IL-1β production[92]. One recent report showed that HIV infection of THP-1 cells enhanced the output of IL-1 in response to various stimuli[73] similarly to the situation in monocytes. Although IL-1α is transcribed at a similar rate to IL-1β, IL-1α mRNA steady state levels are considerably lower, owing to a less stable IL-1α mRNA[101].

The production of two proteins involved in lipid metabolism, i.e. the enzyme lipoprotein lipase (LPL) and the lipid binding protein, apo E, has also been studied in THP-1 cells. Lipoprotein lipase is a glycoprotein enzyme, synthesized by a number of different cells, which is responsible for the hydrolysis of the core triglycerides in triglyceride-rich lipoproteins to glycerol and free fatty acids[79]. Macrophages also secrete LPL[26, 47]. THP-1 cells acquire the capacity to produce LPL after initiation of PMA-induced differentiation[9, 10, 99]. This is unique, since out of all the human leukemia cell lines we tested

(including HL-60, HEL, KG-1, U937) this is the only cell line capable of producing LPL. The THP-1 cell line was furthermore useful to define the molecular basis of LPL regulation in the macrophage. It was shown that the production of LPL is under the control of both the PKC[10] and the adenylate cyclase (Staels and Auwerx, unpublished data) signal transduction pathways. LPL mRNA levels were furthermore influenced by a negative regulatory protein[10].

Apo E is an important component of several plasma lipoproteins[64]. Although the liver is the main site of synthesis, recently apo E has been detected in tissues of various organs including the brain (in the glial cells), adrenals, spleen, ovary, testis and kidney (reviewed in ref. 64). The apo E synthesized by peripheral organs is thought to play an important role in the redistribution of cholesterol between cells and tissues and can tag cholesterol for hepatic clearance in a process called reverse cholesterol transport[64]. Differentiation of monocytes to macrophages is accompanied by an increase in apo E synthesis[110,114]. Macrophage apo E production is furthermore increased by cholesterol loading[13]. The myeloid leukemia cells, HL-60 and THP-1 cells respond to the differentiation process in a similar way to native macrophages, since PMA-induced differentiation of THP-1 cells results in an induction of apo E mRNA[9]. In view of the slow accumulation of the LPL mRNA, we hypothesize that the expression of apo E, instead of being an intermediate stage in the differentiation process (such as the expression of c-fos), is characteristic of the fully differentiated macrophage state. Menju et al.[67] recently used THP-1 cells to study regulation of apo E production by LPS. It was shown that LPS reduced apo E production in THP-1 cells by an inhibition of transcription.

Monocytes, macrophages and atherosclerosis

In most atherosclerotic lesions, and especially those induced by hypercholesterolemia, the first visible abnormality is the attachment of monocyte/macrophages to endothelial cells, and later their migration between them. These monocyte/macrophages localize in the subendothelium and begin to accumulate lipid (reviewed in refs 87, 93). These macrophages ingest and degrade cholesterol-carrying lipoproteins that leak through the endothelium. When they take up more lipoprotein cholesterol than they can excrete, the cholesterol is stored in the cytoplasm in the form of cholesteryl droplets, and finally they become converted into foam cells. By the second month this initial lesion, called 'fatty streak' also contains some smooth muscle cells, which also start to accumulate lipids. The 'fatty streak' keeps on growing and eventually evolves into a 'fibrous plaque', the hallmark lesion of atherosclerosis. As fibrous plaques enlarge, they undergo further changes including calcification, ulceration hemorrhage, and occlusive thrombosis.

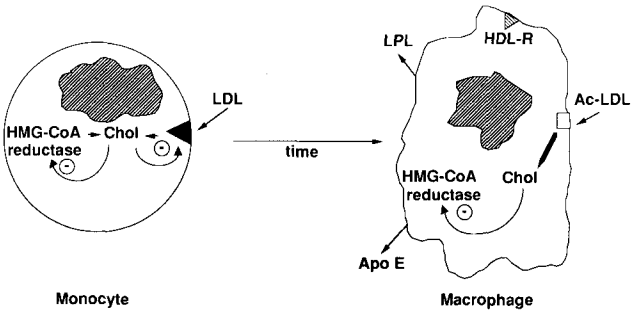

Figure 2. Changes in the expression of genes involved in lipid metabolism during macrophage differentiation. Upon differentiation from a monocyte to a macrophage, the expression of the low density lipoprotein (LDL) receptor is lost. The mature macrophage expresses, however, the scavenger receptor or acetyl-LDL (Ac-LDL) receptor, whose expression is not feedback-regulated by intracellular cholesterol content. The macrophage possesses, furthermore, a receptor for high density lipoprotein (HDL) or HDL receptor. In contrast to the monocyte, the mature macrophage is capable of secreting apolipoprotein E (apo E) and lipoprotein lipase (LPL). Chol stands for cholesterol, HDL-R for high density lipoprotein receptor, and HMG-CoA reductase for 3-hydroxy 3-methylglutaryl coenzyme A reductase. Symbols: black triangle: LDL receptor; shaded triangle: HDL receptor; square: scavenger or acetyl-LDL receptor. —: feedback inhibition.

Although cell proliferation, and particularly smooth muscle cell proliferation, is the principal cellular response associated with the progression of atherosclerosis, the initial lesion is monocyte/macrophage infiltration. Understanding the role of the macrophage in the development of the atherosclerotic lesion is therefore of the utmost importance. When a monocyte differentiates into a macrophage, a remarkable number of changes in the expression of several genes involved in lipid metabolism occurs (fig. 2). In contrast to the monocyte, the mature macrophage expresses the genes for apolipoprotein E[110], for LPL[26,47], for the high density lipoprotein receptor (Auwerx, unpublished data), and for the acetyl-LDL or scavenger receptors[21,49,50,108,109]. The expression of the LDL receptor gene, on the contrary, is almost absent in the fully differentiated macrophage[7,8,43]. The disappearance of the LDL-receptor and the concomitant appearance of the scavenger receptors are important in the process of foam cell formation. Indeed, there is considerable evidence that the accumulation of intracellular lipid is caused by the scavenger receptor family which recognizes negatively charged/modified forms of LDL[21,22,49,50,86,108,109]. In contrast to the LDL receptor, these receptors are not feed-back regulated by cellular cholesterol content. In addition, the cells in the atherosclerotic plaque can all modify LDL in vitro, enabling a better uptake of cholesterol via the scavenger receptor (reviewed in ref. 72). It therefore appears that macrophage differentiation sets the stage for coordinated changes in the expression of several genes involved in lipid metabolism, all leading to an enhanced capacity to accumulate cellular cholesterol, and potentially enhancing foam cell formation and atherosclerosis. The THP-1 cell provides a model for studying these changes in gene

expression. Indeed, all the above-mentioned changes in the expression of genes involved in lipid metabolism have also been observed in PMA-induced differentiation of THP-1 cells[7-10,49,50,67,99,109]. In addition, preliminary experiments (Auwerx et al., unpublished results), suggest that these cells can easily be transformed into foam cells by lipid loading, which makes THP-1 cells also an excellent model to study foam cell formation.

In addition, we used the THP-1 cells to study the regulation of the expression of the LDL-R and HMG-CoA reductase genes by second messengers and a negative regulatory factor. Several compounds which selectively activate PKC, increase intracellular Ca^{2+} concentration, or augment intracellular cAMP concentration, increase the expression of the LDL-receptor and HMG-CoA reductase. This proves that these two genes, in addition to being regulated by cell sterol content[20,30,81,98], are also regulated by second messengers of the phosphoinositol lipid and the adenylate cyclase systems. Since the cAMP, diacylglycerol/PKC and IP_3/Ca^{2+} pathways all lead to protein phosphorylation, it is likely that the distinct phosphorylation events initiated by different messengers may overlap at one or several points, resulting in 'cross-talk' between the different systems[11,107,112]. Furthermore, it was shown that the transcription of the LDL-receptor was induced by cycloheximide, which pointed to the involvement of a labile negative regulatory protein in the regulation of LDL-receptor mRNA levels[7,8]. At present it is unclear whether the regulation of the LDL-receptor by the different second messengers and this negative regulatory protein plays an important role in the in vivo situation or contributes to LDL clearing from the plasma. Considering the magnitude of the changes seen in vitro, it is likely that even smaller changes in the levels of the various second messengers may play an important role in vivo. Activation of transcription could then occur either by blocking synthesis of the negative regulatory protein or by converting it (phosphorylation by second messengers) into a form unable to bind to the positive transcription factor.

Conclusions and future perspectives

In conclusion, after treatment with PMA, THP-1 cells differentiate into a macrophage-like cell which resembles native monocyte-derived macrophages with regard to several criteria such as: 1) morphological characteristics, 2) expression of membrane antigens and receptors, 3) transient induction of several proto-oncogenes, 4) production of several secretory products. Several of these parameters can be used to chart the progress of the THP-1 cell along its differentiation pathway. We therefore believe that the THP-1 cell line represents a valuable model for studying the mechanisms involved in macrophage differentiation and for exploring the regulation of macrophage-specific genes, as they relate to physiological functions displayed by these cells. This cell line can be used to study widely varying aspects of macrophage biology, ranging from the role of the macrophage in host defense, over its role as a secretory cell, to the involvement of macrophages in the pathogenesis of atherosclerosis.

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Abbreviations. apo: apolipoprotein; CFU-GM: colony forming unit for the granulocyte-macrophage; CSF: colony stimulating factor; FcR: IgG Fc receptor; HMG-CoA reductase: 3-hydroxy 3-methylglutaryl Coenzyme A reductase; γ-IFN: interferon; IL: interleukin; LDL: low density lipoprotein; mAb: monoclonal antibody; PKC: protein kinase C; PMA: phorbol 12-myristate 13-acetate; TNF: tumor necrosis factor-α.

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Captivity affects behavioral physiology: Plasticity in signaling sexual identity

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Summary. Little is known about the link between captivity, physiology, and behavior in wild-caught vertebrates. Anecdotal evidence suggests that hormonal changes are responsible for behavioral changes in wild animals brought into captivity. Studying the effects of captivity on reproduction is hampered because wild animals often fail to exhibit sexual behavior under captive conditions. In weakly discharging electric fish, field studies have reported sex differences in electric organ discharges which are rarely seen in the laboratory. I now report the results of a series of laboratory investigations which show that *Gnathonemus petersii* exhibits seasonal, hormone-dependent, phase-specific sex differences in electric organ discharges. Captivity dramatically alters and may even reverse these sex differences as a result of rapid changes in endogenous plasma hormone levels. These findings have broad implications for research on animal physiology and behavior performed in laboratory settings.
Key words. Captivity; electric organ discharge (EOD); sex differences; plasma hormone levels; androgens; estrogen; external morphology; behavioral plasticity.

Introduction

Laboratory research performed on wild animals is typically generalized to naturalistic settings with little regard to the effects of captivity upon the phenomena under investigation. While it is known that captivity has profound effects on both behavior and reproductive physiology in most vertebrates[8, 19, 25–27, 29, 38], the physiological causes underlying the behavioral differences found in field versus laboratory settings have only been surmised. The fact that animals often do not show sexual behavior under captive conditions[19, 25, 29] has made it particularly difficult to study the mechanisms by which reproduction may be inhibited in wild-caught species.

Here I report on a series of laboratory investigations which show robust and replicable captivity effects on the communication of sexual identity in a weakly discharging electric fish, *Gnathonemus petersii.* Newly imported fish exhibit clear hormone-dependent sex differences in the duration of specific phases of their electric organ discharges (EODs). In the laboratory, these sex differences are dramatically altered and may even reverse as a function of profound changes in plasma gonadal steroid hormone levels. Together, these studies provide the first direct evidence of how captivity alters reproductive behavior and its underlying physiology, and explain numerous discrepancies concerning signaling of sex differences in the weakly discharging electric fish.

Laboratory and field studies suggest the EOD of weakly discharging electric fish is used in social communication, species and sexual identification, and possibly as warning signals analogous to the alarm calls of other vertebrates[13–15, 18, 22–24]. Field studies employing relatively small samples have reported largely descriptive, non-statistical accounts of natural sex differences, with considerable variability and overlap between the sexes, in EOD waveform, duration, or pattern of discharge for several species of African mormyrid and South American gymnotiform electric fish species[2, 10, 11]. These field-reported sex differences are rarely observed in the laboratory. Both laboratory and field studies have employed hormone manipulations to induce male- or female-like EODs[3, 6], indicating that these sexual characters are steroid sensitive.

In a previous laboratory study[18], we found a sex difference in EODs of *Gnathonemus petersii,* with males exhibiting shorter EODs and higher peak power spectral frequencies (PPSFs) of the Fourier transformation (fig. 1a). This was surprising because field reports suggested that males of several other mormyrid species have longer-duration EODs and lower PPSFs than females[2, 10] and *G. petersii* administered male hormones exhibited increases in EOD duration and decreases in PPSF[17]. The present studies were designed to further