Macrophage biology: regulation of gene expression

Inflammation occurs when the body suffers aggression either by microbes, traumatisms or a variety of physical agents such as heat, radiation, etc. Inflammation is also involved in the pathogenesis of chronic diseases of auto-immune origin (ie, rheumatoid arthritis) and cancer. In the early stages of this process, there is an increase in the size of the vessels around the inflammatory loci and the release of liquids. After, distinct cells reach these loci in a highly specific order; in the first 24 h neutrophils, at 48 h macrophages, and several days later lymphocytes. Neutrophils destroy most types of microbes. In the initial stages of inflammation macrophages destroy the remaining microbes that escape the neutrophils. In addition, these phagocytic cells remove the apoptotic bodies of dead neutrophils and present antigen to T lymphocytes, thereby initiating the mechanisms of acquired immunity, which ends in the production of antibodies, cytokines and memory cells, the latter a key element for vaccines. Macrophage activity then changes from being pro-inflammatory to being anti-inflammatory, when these cells remove all the tissue debris that arises during healing (Figure 1). Our project is the continued work of many years devoted to the biology of macrophages and dendritic cells. These two cell types are crucial in the innate immune response and form a bridge between innate and acquired immune response.

Macrophages are generated in bone marrow and reach all body tissues through the blood. In normal conditions, a few cells are differentiated in response to certain stimuli and become mature cells or tissue specific cells: dendritic cells, Kupffer cells, microglia, etc, while most are removed by apoptosis. When inflammation occurs, macrophages proliferate, differentiate or become activated under the effect of interleukins or growth factors. When a macrophage becomes activated, it ceases to respond to proliferative stimuli. In certain circumstances, when chronic inflammation is produced, macrophages have a harmful rather than repairing effect, and cause lesions. Our group seeks to determine the molecular mechanisms involved in the proliferation, activation, differentiation and apoptosis of macrophages. Knowledge of these mechanisms could provide therapeutic targets to modulate the activity of these cells during acute or chronic inflammation.

Signal transduction and gene regulation that mediate proliferation, activation and apoptosis of macrophages

One of the best characterised signal transduction pathways is involved in the sequential activation of Ras, Raf-1, mitogen/extracellular signal-regulated kinase (MEK) and the extracellularly regulated kinase (ERK). Activated Raf-1 phosphorylates MEK1 and MEK2 kinases, which in turn activate ERK1 and ERK2. In non-stimulated cells, ERK1 and ERK2 are found in the cytoplasm and relocate to the nucleus after being phosphorylated. Once in the nucleus, they phosphorylate a series of transcription factors. These kinases also participate in the synthesis of nucleotides and in protein translation, both required for proliferation and cellular activation. In macrophages, we have observed that ERK activation is required not only for proliferation, but also for lipopolysaccharide (LPS)-mediated activation, although this activation also blocks proliferation. The duration and time of initiation of ERK phosphorylation determines whether the macrophage proliferates (short phosphorylation) or becomes activated (long phosphorylation). This is explained by the fact that MKP-1, the phosphatase responsible for ERK dephosphorylation, is induced rapidly in response to macrophage colony-stimulating factor (M-CSF) or slowly in response to LPS. In both cases, MKP-1 induction is mediated by protein-kinase C (PKC)ε and is independent of ERK phosphorylation.

We have also reported that Jun N terminal kinase (JNK) activation is required for this induction (Sánchez-Tilló et al, 2007). Furthermore, IFN-γ, which also inhibits proliferation, blocks MKP-1 induction by
M-CSF by elongating ERK phosphorylation (Valledor et al., 2007; Figure 2). Inhibition of MKP-1 induction by RNA interference (RNAi) blocks proliferation and elongates ERK activation. IFN-γ also cross-talks with the MAP kinases (Valledor et al., 2007).

We have cloned the MKP-1 promoter, and by means of luciferase mutations and activity assays, we have localised an AP-1/CRE box which is critical for MKP-1 induction by M-CSF and by LPS. By electrophoretic mobility shift assays and chromatin immunoprecipitation, we have determined that this box is bound by Jun and CREB factors. c-Jun is induced by LPS and M-CSF with the same kinetics as MKP-1 (Figure 3).

Macrophage proliferation is independent of calcineurin but requires immunophilin, without which ERK is inactivated. M-CSF induces the opening of K+ channels, which are required for proliferation (Villalonga et al., 2007).

Our group has devoted many years of research to the study of the regulation of MHC class II molecules. Peptides derived from processed proteins bind to a cleft in the MHC class II molecule surface and are presented to T lymphocytes. Thus, the expression of MHC class II molecules regulates not only the generation of the T lymphocyte repertoire, but also the induction and maintenance of immune response. MHC class gene transcription depends on the interaction and co-operation of several transcription factors which bind to the regulatory elements found in the promoter. However, all the transcription factors de-
Figure 4. Nanoparticles ingested by macrophages.

scribed to date show ubiquitous expression, a finding that does not correlate with the differential tissue expression of MHC class II molecules. A transactivator that does not bind directly to DNA, CIITA (class II transactivator), has been shown to be required for the expression of these genes. We have determined that an AP-1 box acting as an enhancer is responsible for the induction of expression in B lymphocytes and dendritic cells treated with LPS (Casals et al, 2007). Also the upstream regulatory elements interact with the proximal, thereby blocking the transcription. This loop is open when CIITA is present.

In collaboration with Victor Puntes (Institut Català de Nanotecnologia) and Ernest Giralt (IRB Barcelona), we have found that nanoparticles activate macrophages by interaction with the Toll-like receptor4 (Figure 4).

Molecular mechanisms involved in classical and alternative activation of macrophages

Classical or pro-inflammatory activation of macrophages (phenotype M1) is induced by IFN-γ or LPS while activation triggered by IL-4, IL-10 or IL-13 is known as alternative or anti-inflammatory activation (phenotype M2). Apart from a series of structural and functional modifications, the main difference between these phenotypes is the biochemical pathway used for processing the amino acid arginine. IFN-γ or LPS induce nitric oxide synthase 2 (NOS2), which produces nitric oxide (NO), a molecule that has great destructive power and in the first phases of inflammation kills microorganisms. In anti-inflammatory macrophages, arginase is induced and produces proline and polyamines, which catalyse the reconstitution of the damaged extracellular matrix, an event that occurs during the final phases of inflammation. We have found that activation with IL-4 or IFN-γ blocks proliferation in G1/S. However, while the mechanism by which IL-4 inhibits proliferation is p21waf1- and Stat6-dependent, the mechanism used by IFN-γ differs.

Role of TREX1 exonuclease in transcription

We have cloned a protein that corresponds to TREX1 exonuclease. This enzyme catalyses the digestion of DNA in the 3’->5’ direction and shows homology to the TREX2 exonuclease (30%). Genetically modified mice, with a deletion in the TREX1 locus, develop inflammatory myocarditis and have a reduced half life compared to their wild-type counterparts. In humans, mutations in the TREX1 gene have been associated with Aicardi-Goutières Syndrome, a chronic inflammation of the brain, as well as with systemic lupus erythematosus, an auto-immune disease. TREX1 has also been associated with protein members of the SET complex, which digest DNA from cells where apoptosis has been induced by Granzyme A.

In collaboration with experts in crystallography (Ignasi Fita) and in NMR (Maria Macias) at IRB Barcelo-
na, we have determined the structure of TREX1 alone and its binding to DNA (Brucet et al., 2007; Figure 5). TREX1 binds preferentially to certain DNA sequences that correlate with the exonuclease activity. TREX1 has a proline-rich domain not found in TREX2. This domain allows interaction with SH3 or WW domains, which we have demonstrated by NMR and co-immunoprecipitation. These data and the nuclear localisation of the protein have led us to study whether TREX1 is involved in transcription. Also, we have identified a new active histidine that is conserved in DEDDh exonucleases and is required for functional activity (Brucet et al., in preparation).

**Deregulated gene expression in aging**

We are currently testing the molecular changes that occur in the genome of macrophages during aging. By growing macrophages alone in vitro, we eliminate the effects that other cells could exert on them. In addition, we have recently reported that deacetylase activity is required for GM-CSF-dependent functional response of macrophages and dendritic cell differentiation (Sebastian et al., 2007). Since deacetylase activity plays a relevant role in aging in lower organisms, these results have prompted us to study whether it is involved in macrophage aging.

**LXR in neuroinflammation and neuronal degeneration**

LXRs (Liver X receptors, initially discovered in the liver) are members of the nuclear receptor superfamily. Nuclear receptors are ligand-dependent transcription factors that regulate many aspects of development and homeostasis. LXRs are regulated by oxidised forms of cholesterol (oxysterols) and by intermediary products of cholesterol biosynthesis. At the physiological level, LXRs play a crucial role in the positive regulation of genes involved in lipid homeostasis.

We seek to explore whether the activation of LXRs exerts anti-inflammatory and neuroprotective actions in the central nervous system (CNS). As a result of their lack of cellular division and their low capacity to recover from injury, neurons are extremely sensitive to inflammatory processes and immune auto-destruction. For this reason, intervention of the inflammatory process has recently gained attention as a therapeutic strategy to halt the progression of neurodegenerative disorders. Our preliminary studies show that both primary microglia and the microglial cell line BV-2 express the LXRα and β, and RXRα and β isoforms. In BV-2 microglial cells and in primary microglia from neonatal mice, stimulation with endogenous ligands of LXR resulted in the activation of known LXR target genes involved in lipid metabolism. In the mature brain and under physiological conditions, resting microglia serve the role of immune surveillance and host defence. However, these cells are particularly sensitive to changes in their microenvironment and readily become activated in response to infection or injury. Most of the factors released by activated microglia are pro-inflammatory and neurotoxic, thereby contributing to the progression of the neurodegenerative disorder. We have therefore studied the role of LXRs in the regulation of microglial activation in vitro. Our results indicate that a number of pro-inflammatory factors induced by endogenous cytokines are inhibited by LXR agonists (Figure 6). We are currently using microarray technology to establish a more extensive list of pro-inflammatory genes susceptible to down-modulation by LXR agonists and to determine the relative contribution of the macrophage phenotype (M1 vs. M2) in this context.

**Figure 5.** Three-dimensional structure of TREX1.

**Figure 6.** LXR agonists inhibit specific pro-inflammatory responses in microglia activated by IFNγ.
Furthermore, we have also explored the role of the LXR-RXR pathway on programmed cell death in the CNS. The simultaneous use of LXR and RXR agonists resulted in synergistic effects that promote high expression of genes involved in protection against apoptosis in microglial cells, such as the genes Bcl-XL, AIM and NAIP (neuronal apoptosis inhibitory protein). The observation that AIM, an anti-apoptotic factor secreted by macrophages, is also induced in this system led us to propose that this factor mediates paracrine anti-apoptotic actions on other neighbour cells in the CNS, such as astrocytes and neurons. We are currently testing these effects using mixed glial-neuronal cultures and pure neuronal systems. Microarray experiments in pure neuronal cultures are also underway to determine direct effects of LXR agonists on these cells. Our final goal is to establish whether the anti-apoptotic and anti-inflammatory actions of the LXR-RXR pathway can be exploited for the therapeutic intervention of neurodegenerative disorders in vivo.

**Publications**


**Research Networks and Grants**

Anti-inflammatory and anti-apoptotic effects of LXR/RXR agonists in the central nervous system

Marie Curie international reintegration grants, European Commission, 031137: 2006-2008

**Research Director:** Annabel Fernández Valledor

Ayuda para potencializar los grupos de investigación consolidados

Pla de Recerca de Catalunya, 2005SGR 00910: 2005-2008

**Research Director:** Antonio Celada

Nanoparticles as activators of phagocytic cells for the clearance of toxic aggregates of proteins in the brain


**Research Director:** Jorge Lloberas

Programas transcripcionales regulados por LXR en microglia y neuronas: implicaciones en neuroinflamación y neuroprotección


**Research Director:** Annabel Fernández Valledor

Regulation of the expression of genes involved in the proliferation, differentiation, activation and apoptosis of macrophages and dendritic cells


**Research Director:** Antonio Celada

Regulation of the expression of genes involved in the proliferation, differentiation, activation and apoptosis of macrophages and dendritic cells


**Research Director:** Antonio Celada
Collaborations

*Alternative activation of macrophages*
Manuel Modolell, Max Planck Institute (Freiburg, Germany)

*Inflammation and apoptosis*
Joan Maña, Ciudad Sanitaria y Universitaria de Bellvitge (Barcelona, Spain) and Ignacio Umbert, Clinica Corachan (Barcelona, Spain)

*Inflammation and neutrophils*
Victor Asensi, Hospital General de Asturias (Oviedo, Spain)

*Inflammation and polymerases*
Antonio Bernard, Centro Nacional de Investigaciones Cardiovasculares (Madrid, Spain)

*LXR and brain*
Esther Pérez Navarro, Universidad de Barcelona (Barcelona, Spain), Rosa María Sarrias, Hospital Clinic (Barcelona, Spain), Mercedes Ricote, Centro Nacional de Investigaciones Cardiovasculares (Madrid, Spain), Antonio Castrillo, Universidad de Las Palmas (Gran Canaria, Spain), Andrew C Li, University of California (San Diego, USA)

*Signal transduction*
Jin Mo Park, Massachusetts General Hospital (Massachusetts, USA)

*Telomerase and macrophaging*
María Blasco, Centro Nacional de Investigaciones Oncológicas (Madrid, Spain)