[Frontiers in Bioscience 14, 2413-2431, January 1, 2009]

Regulation of monocytes and macrophages cell fate

M. Elba Gonzalez-Mejia[1,2],Andrea I. Doseff [1,2]

[1]Department of Internal Medicine, Division of Pulmonary and Critical Care; Heart and Lung Research Institute; [2]Department of Molecular Genetics, The Ohio State University Columbus, OH, USA

TABLE OF CONTENTS

1. ABSTRACT

Monocytes and macrophages are central cells of the innate immune system, responding to a diverse repertoire of pathogens. These cells originate from a common myeloid precursor in the bone marrow and while sharing responsibilities during innate immunity, differ greatly in their lifespan. Normally, blood monocytes live for just few days before undergoing apoptosis. Macrophages, in contrast, live up for months. Monocytes' lifespan can switch dramatically, from prolonged survival during inflammation to apoptosis as inflammation resolves. Interestingly, many of the mechanisms mediating survival during inflammation and cancer also operate in monocyte/macrophage differentiation. Differentiation and inflammatory stimuli determine monocyte/macrophage lifespan, by blocking the apoptotic pathway and activating a myriad of survival pathways. How these complicated networks of survival and apoptotic regulators are integrated remains yet to be fully elucidated. The present review summarizes the different monocytes' subpopulations and their function during pathogen recognition. We discuss the role of the caspases and the mechanisms that determine monocytes/macrophages fate highlighting their significance in the regulation of inflammatory diseases.

2. INTRODUCTION

The blood consists of a suspension of plasma and cells, corresponding to $1/12^{th}$ of the body weight of an adult (approximately 5-6 L) (1). Blood cells originate from pluripotent hematopoietic stem cells (HSCs) in the bone marrow, which give rise to two progenitors cell lineages, the lymphoid and myeloid stem cells. The myeloid stem cell can differentiate towards megakaryocyte, pre-erythrocytes, myeloblasts, and monoblasts (see in the same issue Droin *et al.* and (2)). In addition, leukocytes (or white cells) are classified based on the presence of granules in granulocytes and agranulocytes. The granulocytes are composed of neutrophils (50-70%), eosinophils (2-4%), and basophils (0.5-1%) totaling normally between 5,000-10,000 cells/mm^3. The agranulocytes are composed of lymphocytes (20-40%) and monocytes (3-8%), totaling 300-700 cells/mm^3. The leukocytes play a fundamental role in the immune system by responding to a diverse repertoire of pathogens including bacteria, viruses, parasitic, fungal infections, and in some pathological conditions against the host cells (3). Several steps control the number of hematopoietic cells. In one hand, stem cells proliferate giving rise to different cell lineages in the presence of the appropriate differentiation factors.

Moreover, once cellular identity is achieved, a well-coordinated balance between survival and cell death pathways determines leukocyte's fate.

Monocytes and macrophages are central components of the innate immune system, that are responsible for the recognition of the inflammatory stimuli, the initiation of the inflammatory response that is characterized by the production of proinflammatory cytokines, and the clearance of the insult allowing the resolution of inflammation. Similarly to other hematopoietic cells, monocytes and macrophages' lifespan is determined by the homeostatic balance between survival and apoptotic pathways. Intense investigation in this area in the last decade revealed a complex network, far from being completely understood. But, with new tools available, a more detailed characterization has identified different subpopulation of monocytes and macrophage that seem to have distinct functional patterns of activity. In this review we will focus on the complex networks and molecules that control monocyte/macrophage cell fate and the regulation of the major pathways.

3. MONOCYTES

Monocytes and macrophages originate in the bone marrow from a common progenitor, the colony-forming unit macrophage (CFU-M). The colony-stimulating factor-1 (CSF-1) promotes the differentiation of the CFU-M into an intermediate stage named "monoblast", which in turn differentiates into a "promonocyte". What determines a promonocyte stage has not yet being fully characterized but this cell will give origin to monocytes (4). These different stages were characterized based on their size, nuclear/cytoplasmic ratio, granularity, and shape (5). The life span of the cells in the bone marrow has not been defined, but once they reach the bloodstream they live for a short period of time. Pioneer work from van Furth determined, using radiolabeled cells *in vivo*, that the majority of the monocytes circulate in the bloodstream for 48 hr and only a small fraction (15%) remained alive for up to 168 hr (6). After this period of time, circulating monocytes undergo spontaneous apoptosis or programmed cell death (PCD) (7). Monocytes can escape their apoptotic fate, by migrating to different organs and differentiating into tissue macrophages, processes that will be described in detail in the following sections.

Monocytes express specific receptors responsible for pathogen recognition (8). Recently, Auffray and others demonstrated using mice expressing GFP-labeled-monocytes that circulating monocytes present a "patrolling behavior", allowing the fast recognition of pathogens and rapid recruitment to damage tissue (9, 10). In addition, their rapid response is accompanied by their ability to produce inflammatory cytokines including between others interleukin-1 beta (IL-1β), IL-8, IL-10, tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and growth factors like macrophage-colony stimulation factor (M-CSF) and granulocyte macrophage-colony stimulation factor (GM-CSF) (11). These cytokines act in autocrine and paracrine fashion increasing monocyte life span by blocking PCD prolonging their survival and their accumulation in tissues (12-15). In addition, the persistence of activated monocytes at sites of inflammation is mediated by the formation of immune complexes (IC) triggering secretion of several inflammatory cytokines that also contribute to monocyte accumulation (16). Monocytes exposed to ICs, showed the activation of the PI3K/AKT survival pathway and the inhibition of spontaneous apoptosis (16). In chronic inflammatory diseases such as rheumatoid arthritis (RA) and atherosclerosis, the persistent high levels of inflammatory cytokines and the presence of ICs constitute critical factors mediating the excessive infiltration of monocyte/macrophages contributing to the persistence of inflammation (17).

3.1. Subpopulations of monocytes

In the early sixties, van Furth and colleagues described the presence of two pools of monocytes (6). Studying the kinetics of monocytes' fate in mice, they found that the majority of the monocytes remained in circulation, whereas a small pool of monocytes was randomly able to leave the circulation and migrate to specific tissues becoming macrophages (18). Based on the expression of the cluster of differentiation (CD) known also as the human leukocyte differentiation antigens (HLDA), monocytes are referred as $CD14^+$ cells due to their ability to express the lipopolysaccharide (LPS) binding protein receptor CD14 (19). With the use of flow cytometry analysis (FACS) and two-color immunofluorescence, a new subpopulation of monocytes was identified due to the expression of the low affinity Fcγ receptor (FcγRIII) or CD16 (20, 21). Based on these findings, monocytes are currently classified into two groups the "classical" or $CD14^+CD16^-$ and the "non-classical" or $CD14^+CD16^+$. The classical subpopulation represents 95% of the circulating monocytes in healthy individuals, whereas the non-classical comprised the remaining 5% (20). Experiments in human and mouse cells stimulated with LPS showed that both populations produced similar levels of IL-1β, TNFα, and IL-6, but the $CD14^+CD16^-$ produce higher levels of IL-10 (22-24).

Two subpopulations can be also characterized within the non-classical depending on the level of CD14 expression, the $CD14^{high}$ $CD16^+$ and the $CD14^{dim}$ $CD16^+$ comprising 4.7% and 0.8% respectively of total circulating monocytes. These two groups differ in their size, $CD14^{high}$ $CD16^+$ are larger than the $CD14^{dim}$ $CD16^+$ (18.4 and 13.4 μm respectively) (20). In addition, they share the same repertoire of cell surface expression molecules (CSEM) but differ in the expression level of some of them (Table 1) (20, 22, 24-27). The $CD14^{high}$ $CD16^+$ express high levels of ICAM-1 and VCAM-1 (20). On the other hand, $CD14^{dim}$ $CD16^+$ express low levels of TLR2, producing high levels of TNFα but low IL-10 upon LPS stimulation (24). These characteristics suggest that $CD14^{dim}$ $CD16^+$ are the major producers of TNFα during inflammation (24, 28). Studies in sepsis patients revealed increased numbers of $CD14^+CD16^+$ in PBM and high levels of IL-10, IL-6, and TNFα (25). Similarly increased in the $CD14^{dim}$ $CD16^+$ was reported in patients with lung injury (29). Further analysis from chronic colitis, atherosclerosis, and sepsis patients showed that the increased number of $CD14^+CD16^+$

Table 1. Cell Surface Expression Molecules (CSEM) and their function

FUNCTION	CSEM	$CD14^{+}CD16^{-}$	$CD14^{high}CD16^{+}$	$CD14^{dim}CD16^{+}$	Macrophages
Activation	CD14	high	high	low	-
	CD16	-	+	+	+
	TLR 4	+	+	+	+
	TLR 2	low	high	low	+
	MHC-I	high	high	high	+
	MHC-II	-	-	-	+
	HLA-DR	low	high	high	+
	FcγRI	+	-	-	high
	FcγRIII	-	+	+	high
	CD86	low	high	high	low
	MR	-	-	-	+
	ScR	-	-	-	+
Adhesion	CD11a	low	-	-	high
	ICAM-1	-	+	+	low
	VCAM-1	-	+	+	low
Migration	CCR2	+	-	-	-
	CD62L	+	-	-	-
	CX_3CR1	low	high	high	low

$^{+}$ Constitutively expressed; $^{-}$ Absent; ^{high} Highly expressed; ^{low} Lowly expressed

corresponded just to an elevated level of the $CD14^{dim}$ $CD16^{+}$ subpopulation (25, 30, 31). Together, these data support the importance of this small subpopulation in the physiopathology of inflammatory diseases. While the mechanisms mediating such response are not yet fully understood, it may suggest different migratory properties of these subpopulations.

3.2. Properties of monocytes

The central function of monocytes is innate immune-surveillance. Circulating monocytes can migrate to tissues differentiating into macrophages. The migration is a multi-step mechanism that involves adhesion and extravasation, mediating the localization of monocytes to the specific tissues, and culminating with their differentiation into macrophages. Initially, it was proposed that the migration of monocytes from the peripheral blood to the tissues was random (6). However, it is by now well accepted that monocytes present migratory properties depending on the expression of leukocyte adhesion molecules on their surface and their ability to move upon a gradient of chemoattractant factors (13, 32). The mechanisms involved in adhesion, were initially analyzed *in vitro* in isolated venous and arterial endothelial cells (EC) co-cultured with monocytes. Pretreatment with different cytokines, showed that monocytes' attachment is cytokine-dependent and clearly mediated by the expression of numerous members of the LAM (leukocyte adhesion molecules) family (33). $CD14^{+}CD16^{+}$ monocytes express ICAM-1 or CD11b (intracellular adhesion molecule 1) and VCAM-1 or CD49 (vascular cell adhesion molecule-1), which mediate their interaction with the vascular endothelium (33) (Figure 1). Treatment with IL-4 or IL-1β induces increased expression of ICAM-1 and VCAM-1 in monocytes, promoting their interaction with EC (34). Furthermore, blocking antibodies for ICAM-1, LFA-1 (lymphocyte function-associate antigen) or CD11a prevented their adhesion (35). Thus, cytokines facilitate the adherence of monocytes to EC by regulating the expression of these receptors. In the case of the classical $CD14^{+}CD16^{-}$, the interaction with EC is facilitated by the expression of CCR2 and CD62L (the chemokine receptor and leukocyte adhesion molecule respectively (36) and Figure 1). During inflammation, a complex network of cytokines elicited by the monocytes and the damaged tissues contribute to their migratory process. Monocytes induce MCP-1 (monocyte chemoattractant protein-1) which mediates their recruitment to the tissues (37). On the other hand, tissues produce fractalkine (FNK) (38), attracting the monocytes expressing the fractalkine receptor (CX_3CR1) (12, 39). The second step in migration is extravasation, which involves the trafficking of the monocytes from the blood vessel to the tissue. $CD14^{+}CD16^{-}$ express low level of the fractalkine receptor, CX_3CR1^{low}. In contrast, $CD14^{+}CD16^{+}$ express high level of fractalkine receptor, CX_3CR1^{high} (10, 40). Hence, it is recognized that specific subpopulations of monocytes characterized by defined surface expression molecules, will provide the source for resident or recruited tissue macrophages found at sites of inflammation (Figure 1).

The mouse and human CD14 share high level of sequence homology (41). In addition, mouse monocytes express Gr-1, a cell surface protein belonging to the Ly-6G "lineage-restricted proteins on lymphoid cells". Based on the expression level of Gr-1 mouse monocytes can be also divided in two populations: $CD14^{+}Gr\text{-}I^{+}$ and $CD14^{+}Gr\text{-}I^{-}$ (42). Like human monocytes, these populations express the fractalkine receptor CX_3CR1. Thus, it has been suggested that the human $CD14^{+}CD16^{-}CX_3CR1^{low}$ are equivalent to the mouse $CD14^{+}Gr\text{-}I^{+}CX_3CR1^{low}$ and the $CD14^{+}CD16^{+}CX_3CR1^{high}$ to the mouse $CD14^{+}Gr\text{-}I^{-}CX_3CR1^{high}$ (10, 43). With the used of adoptive transfer techniques and GFP-labeled cells, it was showed that $CD14^{+}Gr\text{-}I^{+}CX_3CR1^{low}$ have a shorter life span and migrate faster under inflammatory conditions so they have been recognized as the "inflammatory subset", whereas the $CD14^{+}Gr\text{-}I^{-}CX_3CR1^{high}$ are considered the "resident" monocytes (10). Therefore, the migratory properties have been suggested to be most likely due to the different expression of CX_3CR1 (9, 38, 43).

4. MACROPHAGES

The realization that macrophages originate from monocytes was described first by van Furth (5). However, the term macrophage was defined almost 100 years ago by

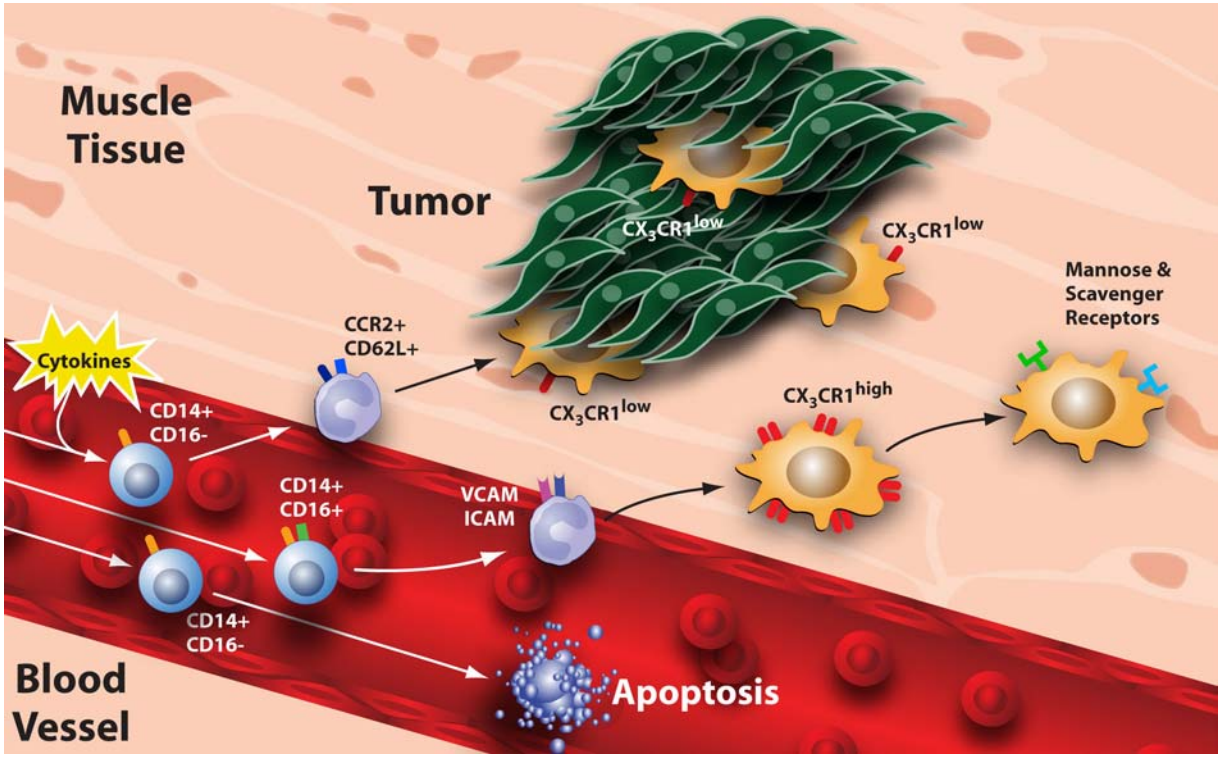

Figure 1. Model of monocyte migration and differentiation into macrophages

Aschoff (44) to describe a large phagocytic mononuclear cell. Macrophages have been classified as the most specialized cell in the phagocytic system and take their named depending on their tissue localization (45-47). They are called Kupffer cells in the liver, alveolar macrophages in the lungs, histiocytes in the connective tissues, osteoclast in the bones, microglia in the brain, and Langerhans cells in the skin (48). Sometimes their named is based on the organ where they are found such as lymph node, thymus, gastrointestinal, genitor urinary, red pulp, endocrine, pleural, epithelial or peritoneal macrophages. Macrophages are highly heterogeneous and can express different cell surface receptors, even within the same organ, making the understanding of these cells even more challenging (47). Regardless of their localization, macrophages are markedly different from monocytes in their longer life span, ranging from months to years (7, 48). The pathways and proteins that confer this longer survival are not fully characterized, but their identification and recognition will evidently provide important tools to potentially manipulate macrophage accumulation.

4.1. Monocyte to macrophage differentiation

The process of monocyte to macrophage differentiation is initiated once monocytes reach the target tissue. Monocytes can differentiate into tissue macrophages, dendritic cells, and osteoclast (49). This review will focus only on monocyte to macrophage differentiation. GM-CSF, M-CSF, and CSF-1 can meditate the differentiation process. These differentiation stimuli activate survival pathways leading to changes in gene expression, which ultimately determine phenotypic changes and specify the expression of macrophage-associated cell surface antigens. Animal models have been instrumental to study the differentiation process. GM-CSF deficient mice for example develop normally and have normal hematocrite and leukocyte numbers but develop lymphoid hyperplasia in the lungs (50). On the other hand, M-CSF deficient mice are smaller, have a lower body weight, and show extensive skeletal deformities due to a reduction in the number osteoclasts. Interestingly, these animals have severely reduced number of blood monocytes, peritoneal macrophages, and tissue macrophages but present normal hematocrite's numbers. In addition, M-CSF deficient mice develop osteoporosis. Thus, these findings suggest that M-CSF has a fundamental effect on the myeloid progenitor cells that give raise to monocytes and macrophages (51, 52).

In vitro studies with primary monocytes and human myelomonocytic leukemia cell lines such as U937, HL-60, and THP-1 have been extensively used to study differentiation. These cell lines in the presence of M-CSF, GM-CSF, or retinoic acid (ATRA) differentiate into macrophages (26, 53, 54). Human monocytes and bone marrow macrophages cultured in the presence of M-CSF can live up to 28 days, increasing in size from 11.6 to 22.4 µm and developing the classical macrophage phenotype (55-57). The complete morphological characteristics of a mature macrophage are acquired during the first 5-7 days of differentiation. After 48 hr GM-CSF-treated monocytes and bone marrow macrophages show a decrease in CD14 expression and an increase in CD11b, scavenger receptor (ScR), and mannose receptor (MR or CD206) (26, 58). In

fact, MR is the best characterized marker of all mature monocyte-derived macrophages showing high sequence homology in vertebrates (59-62).

M-CSF and GM-CSF binding to their receptors induce the activation of survival signaling pathways (see Hunter *et al.* in this issue for a comprehensive review). One of the most extensively studied kinases activated during this process, is Protein Kinase B (PKB or AKT) (see Rane and Klein in the same issue). M-CSF stimulation of monocytes activates the PI-3K/PDK1 pathway promoting the phosphorylation/activation of AKT. Treatment of monocytes with the PI3K inhibitor LY294002, results in an inhibition of AKT and monocyte/macrophage differentiation (54). GM-CSF or ATRA treatment of monocytes and myelomonocytic cells, results in the activation of the MAPK/ERK/MEK signaling pathway by promoting the phosphorylation of Mitogen-Activated Protein Kinase (MAPK), Extracellular signal-Regulated Kinase (ERK1/2), and MAP-ERK-kinase (MEK1) and the activation of the transcription factors such as NF-κβ and signal transducers and activators of transcription (STAT) family members (53, 63).

Gene expression analysis of M-CSF or GM-CSF-differentiated monocytes showed an increase in the expression of several genes (64-66) such as the transcription factor PU.1, a member of the transformation-specific genes *ets* family and surface receptors such as MHC-II, FcγR and ScR, between others (65, 67). Consistent with its role in differentiation, $PU.1^{-/-}$ mice show decrease lymphoid and myeloid precursors (68). ScR expression is regulated by PU.1 so it is conceivable that ScR expression increases accompanying the increase of PU.1 expression during differentiation (27). Microarray analysis of monocytes and myelomonocytic cells stimulated with TPA show a dramatic increase of Hsp27 (66). In agreement with these results we found a forty-time increase in the expression of Hsp27 protein during monocyte/macrophage differentiation (69). In contrast, the expression of genes involved in signal transduction and apoptosis, such as calreticulin and PKCδ were downregulated during differentiation (64). Thus, the differential gene and protein profiles help to identify the similarities and differences of monocytes and macrophages (64, 65).

Comparative proteomics analysis of monocytes and alveolar macrophages show that monocytes express high levels of cytoskeleton proteins related with phagocytes and chemotaxis, such as serine protease, leukocyte elastase inhibitor, cofilin, F-actin capping protein β (70). Whereas the alveolar macrophages express high levels of cathepsin B, Hsp27, aldehyde dehydrogenase, pyruvate kinase, aldolase A, phosphoglycerate mutase I, superoxide dismutase and peroxiredoxin. These findings suggest the specialized function of alveolar macrophages in phagocytic functions (70). Interestingly, comparative analysis between healthy mammary macrophages and TAMs (Tumor Associated Macrophages) found in the mammary tumor, show different cell surface protein expression and changes in the morphology including size, density and tumoricidal effect distribution (71). Thus, these findings suggest that gene profile in tissue specific macrophages is determined by the combination of cytokines, adrenergic, and cholinergic agonists, hormones and immunoglobulins found in the tumor microenvironment (47, 71, 72).

An important regulator of monocyte to macrophage differentiation is the cyclin inhibitor p21 which is known to control cell proliferation. Myelomonocytic cells induced to differentiate with TPA show increased p21 mRNA (73). Furthermore, p21 stably transfected myelomonocytic cells arrest at G0/G1, resulting in a more differentiated stage and express higher levels of CD11b (74). In the other hand, myelomonocytic cells stably transfected with antisense-p21 have reduced levels of differentiation when treated with ATRA (74). Interestingly, p21 knockout mice have normal numbers of monocytes and macrophages, This effect may be due to the fact of redundancy of p21 with other cell cycle dependent regulators (75). However, when challenged intraperitoneally with serum from RA mice the p21 deficient mice failed to develop RA, lacked monocyte accumulation in synovial fluids and had no cartilage or bone erosion (76). Interestingly, cytometry analysis of monocyte subpopulation, showed that only the $CD14^{+}Gr\text{-}1^{+}$ CX_3R1^{low} (inflammatory subset) was decreased, whereas the resident subset of $CD14^{+}Gr\text{-}1^{-}$ CX_3R1^{high} was not altered (76). Thus, these findings suggest that while *in vitro* p21 has a role in cell-cycle arrest facilitating monocyte/macrophage differentiation, while *in vivo* p21 seems to be responsible in their migratory properties during inflammation (74, 76).

4.2. Functions of macrophages

Initially macrophages were described as large phagocytic cells able to "eat" (77). Currently, is well known that their function also includes antigen presentation, mainly due to the MHC responsible for recognition of self and non-self molecules. This property allows macrophages to initiate the primary inflammatory response and their participation in tissue remodeling. In addition, macrophages are also responsible in the phagocytosis of apoptotic cells, a process clearly non-inflammatory (78). The phagocytic functions are mediated by an extensive repertoire of receptors, which identity and mechanisms of function continue to be an area of intense investigation (61).

4.2.1. Antigen presentation

Macrophages initiate cell-mediated immune response against pathogens. The pathogen recognition pattern is mediated by the MHC chains, leading to the formation of the "MHC-peptide complex" that initiates the inflammatory response (79). The MHC are classified in class I and II, based on the structure of their extracellular chains, also referred as C1a or HLA-DR and CD38 or HLA-DQ respectively (80). MHC-II presents two extracellular chains α and β forming a four-domain structure (α1, α2, β1, and β2) (81). Monocytes express MHC-I, but during differentiation MHC-I expression is inhibited while MHC-II is induced (82). MHC-II expression is regulated by the coactivator class II

transactivator (CIITA). The expression of this transactivator is regulated by a complex mechanism. The expression of the CIITA gene can be controlled by three independent promoters (pI, pIII, pIV). While pI and pIII drive CIITA expression in dendritic and B cells respectively, pIV is responsible for regulating its expression in macrophages. Consistently, deletion of pIV resulted in selective depletion of MHC-II in macrophages (83). Moreover, pIV conditional knockout mice develop various inflammatory and autoimmune syndromes, due the loss of MHC-II-mediated antigen presentation (83).

4.2.2. Phagocytosis

Macrophages are the most specialized cells of the phagocytic system, representing a major defense mechanisms against a wide variety of microorganism, including virus, fungi, protozoa, and bacteria. Furthermore, they are also able to phagocytose apoptotic cells contributing to the clearance of defective cells promoting tissue repair and remodeling. The recognition of pathogens and cells is mediated by specific macrophage surface receptors (84). During apoptosis, dying cells change membrane symmetry exhibiting a new range of extracellular molecules (85). Probably the best characterized is the exposure of the phosphatidyl serine (86), in a process that depends on caspase-3 activation (87). The recognition of apoptotic bodies by macrophages is mediated by specific receptors including the phosphatidylserine receptor (PSR), the scavenger receptors, the ATPase membrane transporter ABC1 and one of the tyrosine kinase receptor Tyro3 (see Curtis *et al.*, in this issue and (87-90)). Mice lacking ABC1 accumulate apoptotic bodies during development (90). Thus, these molecules allow the macrophage to differentiate between alive and death cells.

Lipopolysaccharide and polysaccharides present in pathogens' cell walls are recognized by macrophages by the MHC-II, the TLR, MR, ScR and FcγR (91-93). These specific signals once recognized allow the formation of the "peptide-complex". The receptor-peptide complex coordinates the reorganization of the cytoskeleton and recruitment of several molecules such as lectins, N-acetylglucosamine, and the integrins receptor vitronectin (94, 95). Following the internalization, the target-peptide is enclosed in the phagosome, a plasma membrane-surrounded-vesicle (96). Soon after its formation, the phagosome modifies its phospholipids composition, acquiring hydrolases and lysosomal proteins that contain N-acetylglucosamine-1-phosphate (GlcNAc-1-P) linked to serine residues (97). In the phagosome, apoptotic bodies are opsonized suppressing the transcription and translation of inflammatory cytokines (78). The phagosome-containing pathogens acidified (lower pH) and lysosome proteins are recruited onto the phagosomes inducing the production of hydrogen peroxide (H_2O_2) and reactive oxygen species (ROS) that act as a killing mechanism (98). In turn, H_2O_2 induces the release of macrophage-inflammatory protein (MIP-1α and -β), and monocyte chemotactic protein-1 (MCP-1), cytokines that contribute to the inflammatory process (99). Once phagocytosis is finished, the phagosome moves and re-integrates into the cellular membrane (100) (see Curtis *et al.* in the same issue for an extensive review in phagocytosis). Interestingly, while many parasites are clear rapidly by phagocytosis, others such as in the case of Chagas's disease in this case the *Trypanosome cruzi*, remains inside the phagosome for a long time (101). These intracellular parasites inhibit the apoptotic mechanism, contributing to development of chronic inflammation (101, 102).

4.2.3. Repair and remodeling

The initial evaluation of the role of macrophages was conducted in mechanically-damage tissue. Mice exposed to abdominal-tissue damaged, repaired tissue faster inhibiting the formation of peritoneal adhesion, when peritoneal macrophages were applied intraperitoneally (103). After abdominal surgeries there is a high incidence of "peritoneal adhesion" contributing to post-surgery complications. Interestingly, animal models are extensively utilized to study this mechanism. The "macrophage Fas-induced apoptosis" or MAFIA model carries two copies of the FasR linked to a myristylated-targeting peptide able to induce, when the cells are exposed to AP29187, the trimerization of the intracellularly tail of the death receptor (104). Upon trimerization cells undergo apoptosis due to the activation of caspase-3. In the MAFIA mice the FasR-chimera was clone under the control of the macrophage specific receptor c-fms (CSF-1). Hence, this model provides a system to conditionally ablate macrophages when exposed to the AP29187 compound. In contrast to wild-type animals, MAFIA mice treated with AP20187 show peritoneal adhesions post-surgery (61). These findings support the important role of macrophages during tissue repair and remodeling. Hence, understanding the role of macrophages in more detail can be a great tool in developing new approaches for tissue repair.

5. MONOCYTE AND MACROPHAGE CELL FATE

Cell fate is determined by the balance between survival and apoptotic pathways. In monocytes, a constitutively activated cell death program seems to support their short life span. Notably, monocytes' life span has great plasticity, as mentioned in above sections, prolonged survival is triggered by malignant transformation or during inflammation (105), but survival is promptly reverted as inflammation resolves. In contrast, macrophages have acquired mechanisms that inhibit the apoptotic program and activate survival pathways responsible for promoting a longer live span. The distinct molecules that regulate the changes in cell fate have been much studied in the last decade providing a complex repertoire of proteins responsible for cell fate determination. In this section, we will discuss general mechanisms that control monocytes and macrophages life span.

Programmed cell death (PCD) or apoptosis is an evolutionary conserved mechanism essential for normal development and provides a cellular defense against pathogens (106). Importantly, apoptosis participates in the control of cell number during maturation of the immune system and in the resolution of the immune response (107).

Apoptosis was originally recognized by a group of distinct morphological changes, such as nuclear fragmentation, cytoskeleton disruption, cell shrinkage, and membrane blebbing which then lead to the fragmentation of the dying cell into apoptotic bodies that are recognized and engulfed by macrophages (88, 105). In addition, apoptosis involves the biochemical activation of the apoptotic machinery composed by a well-conserved group of cysteine-proteases, the caspases (108). Fourteen caspases have been so far identified (109). These proteins are constitutively expressed as inactive precursors that become proteolytically active upon apoptosis. The founder member of the family, caspase-1, was identified in monocytes being responsible for cleaving the inflammatory cytokines IL-1β and IL-18 (110). Caspase-1 knockout mice show decrease inflammation when infected with bacteria or LPS (110, 111). By now the role of caspase-1 in regulation of inflammation is well recognized. In addition, caspase-4 and -5 have also been involved in inflammation (112).

Based on their position in the apoptotic cascade, the caspases are classified in "initiators" (caspase-1, -2, -8, -9, and -10) and "executioners" (caspase-3, -7, -6). Initiator caspases possess a long N-terminal prodomain containing protein-protein interaction motifs; caspase-1, 2, 4, 5, and 9 contain the caspase recruitment domain (CARD). Whereas caspase-8 and -10, contain the death effector domains (DEDs) (113). These domains participate in the activation of the caspases by providing protein-protein interaction. In contrast, the executioners or effector caspases have a short amino-terminal prodomain that is not evolutionary conserved. As the role of this domain remains unclear, the regulation of the executioner caspases by interacting proteins is still elusive. Recently, we show that Hsp27 binds to the amino-terminal domain of caspase-3, inhibiting its second proteolytic cleavage (69). Whether the prodomains of the other executioner caspases are able to provide docking sites for inhibitors remains to be proven.

Apoptosis can be activated by two main pathways, the death receptor or "extrinsic" and the mitochondria-mediated or "intrinsic" pathways. The extrinsic pathway is triggered by the binding of ligands to the death cell surface receptors belonging to the TNF-receptor family (114). In monocytes the extrinsic pathway is modulated by two major receptors belonging to the TNF family, the CD95 or Fas receptor (FasR) and the Tumor Necrosis (TNF)-Related Apoptosis-Inducing receptors TRAIL-R1 and TRAIL-R2 also named DR4 and DR5 respectively (115, 116). Activation of these receptors promotes the recruitment of the cytoplasmic adaptor proteins TRADD and FADD that in turn, activate caspase-8 (117). In the intrinsic pathway, apoptotic stimuli induce changes in the mitochondria membrane permeabilization, allowing caspase-9 activation (118). Different molecules are involved in the regulation of mitochondrial permeabilization, providing a homeostatic balance that contributes in cell fate determination. Importantly, independently of how apoptosis is initiated all pathways converge en the activation of the executioner caspases, which are responsible for cleaving proteins of diverse biological function from transcription factors to kinases and phosphatases. The caspase-mediated proteolysis is essential for the formation of apoptotic bodies. As the apoptotic process must be tightly regulated, it is postulated that multiple checkpoints contribute to the proper regulation of the caspase cascade.

5.1. Pro-apoptotic regulators

In monocytes, the trimerization of the death receptors leads to the recruitment of adaptor proteins. FasR recruits the cytoplasmic adaptor protein "Fas Associated Protein with Death Domain" (FADD) and TRAIL-R1/2 the cytoplasmic adaptor protein TNF receptor-associated protein (TRADD) (117). Recruitment of these proteins forms the "DISC complex", composed by FasR/FADD/caspase-8 or TRAIL/TRADD/capase-8 respectively (116, 119) (Figure 2). FADD and TRADD contain two Death Domains (DED) able to recruit other DED-containing proteins (117). For example, caspase-8 and caspase-10 interaction with FADD and TRADD through DED–DED domains leads to their autoproteolytic activation (113, 120). Activated caspase-8 can either directly or indirectly activate caspase-3 (116, 121).

The inhibition of the cognate FasR/FasL in monocytes' cultures results in the inhibition of monocyte spontaneous apoptosis (122). The functional role of Fas *in vivo* was evaluated using the FasR (lpr/lpr) and FasL (lpr/gid) knockout mice. Both mice have increased numbers of inflammatory and resident subsets of monocytes ($CD14^{+}Gr\text{-}1^{+}CX_3CR1^{low}$ and $CD14^{+}Gr\text{-}1^{-}CX_3CR1^{high}$ respectively), resulting in lymphadenopathy, splenomegaly, and in accumulation of macrophages in the tissues including lung, liver and spleen (115, 123). Together, these findings highlight the importance of the Fas-mediated pathway in monocyte/macrophage cell fate.

The role of caspases *in vivo* has been more difficult to study, caspase-8 and caspase-9 knockout homozygous embryos mice are non-viable showing neural tube defects, while heterozygous embryos, can survive up to 4–5 weeks but present phenotypes that vary in severity (124, 125). Caspase-3 knockout mice, has severe development defects and die early in life, suggesting that caspase-3 is an essential caspase in apoptosis and has also functional roles in development (126). Future work in conditional knockouts may provide specific knowledge in the role of these caspases in monocyte/macrophage life span.

The intrinsic pathway is activated normally in response to stress or death stimuli, such as DNA-damaging agents (127). Upon stress cytochrome c and Smac/Diablo are released from the mitochondria. Cytochrome c in the presence of ATP binds to the apoptotic protease activating factor 1 (Apaf-1) and caspase-9 forming the "apoptosome". The apoptosome, was first identified in monocytic leukemia cells, induces caspase-9 activation followed by cleavage of caspase-3 (128, 129). Smac/Diablo forms a complex with XIAP relieving caspase-3 form the inhibition of XIAP (130, 131) (Figure 2).

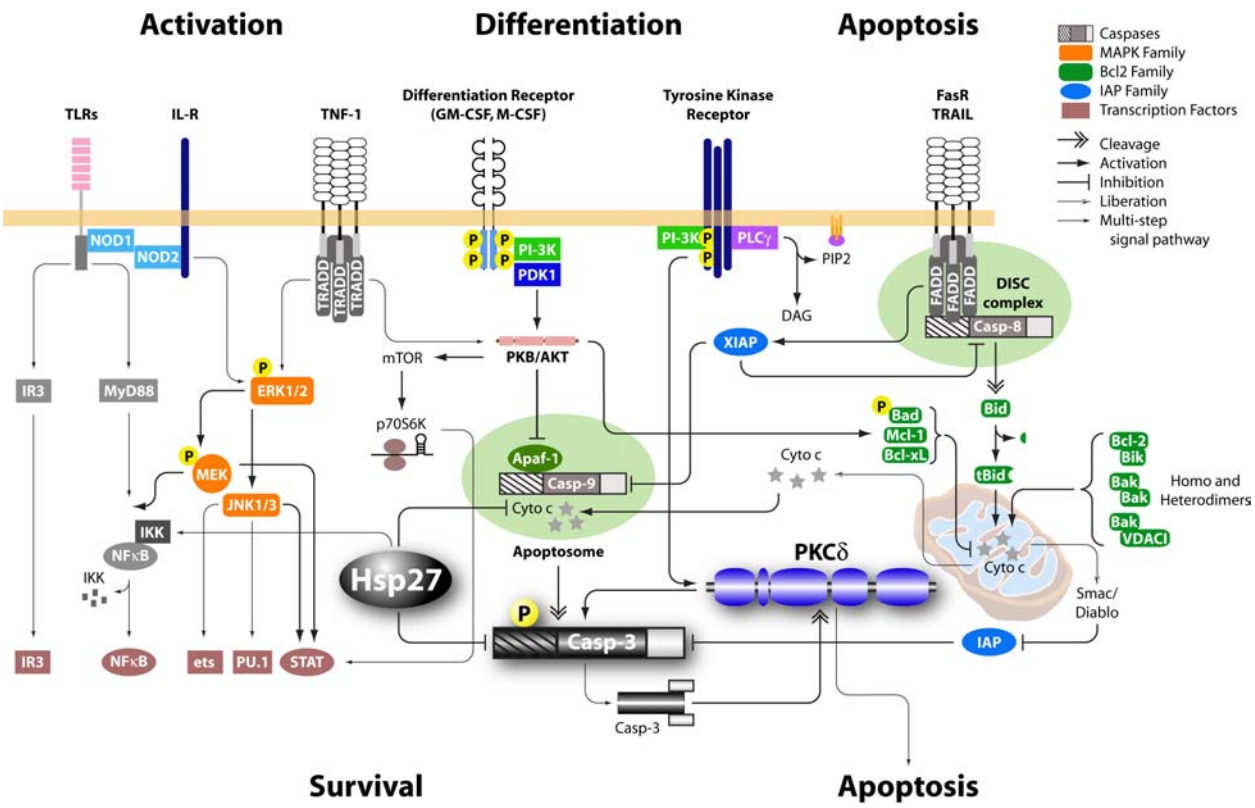

Figure 2. Signal pathways involve in monocytes and macrophages cell fate

The Bcl-2 (B-cell lymphoma 2) family is composed by a large number of homologues which act as pro- or anti-apoptotic regulators, which modulate release of cytochrome c and Smac/Diablo release from the mitochondria to the cytoplasm (118, 132) (Figure 2). This family includes, the anti-apoptotic proteins Bcl-2, Bcl-xL, A1, Bcl-w, Mcl-1 and pro-apoptotic members. The pro-apoptotic family members are classified based on the number of conserved Bcl-2 homology (BH) domains. One group composed by the Bax family includes Bak, Bax, and Box, have three BH3 domains. The second group corresponds to the BH3-only domain proteins. This group includes Bad, Bid, Bik, Bmf, Bim, Hrk, Noxa, and Puma (133). Thus, Bcl-2-proteins act as "guardians" regulating in pairs cellular fate. The function of the Bcl-2-like proteins can be regulated through phosphorylation, dimerization, transcriptional regulation, proteolytic cleavage, and cellular localization (134).

In myelomonocytic leukemia cells, the presence of Bcl-2, Bcl-xL, and Mcl-1 was showed to contribute to cell survival. Overexpression of Bcl-2 and Bcl-xL in monocytic cells increases the protection against apoptotic stimuli. Bcl-2 protective activity is mediated by phosphorylation via AKT. Bcl-2 phospho-negative mutants show a dramatic decrease in the ability to protect myelomocytic cells during flavopiridol or TRAIL-induced apoptosis (135, 136). The presence of Bik, Bak, Bax, Bad, and Bid has also been reported in the monocytic lineage. Subcellular localization of these proteins, from the cytoplasm to mitochondrial membrane, seems to promote apoptosis (137, 138). Mcl-1 expression decreases in cells treated with flavopiridol, resulting in an increase of cell death in myelomonocytic leukemia cells (141). In addition, Mcl-1is overexpressed in tissue macrophages isolated from RA patients whereas the silencing of Mcl-1 resulted in the induction of apoptosis (141, 142). The expression levels of some of the Bcl-2 family members can be transcriptionally regulated by NF-κB (133, 139). In agreement with this, blocking of NF-κB with the specific inhibitor PDTC (pyrrolidine dithiocarbamate) in primary macrophages or a macrophage-like cell line (RAW), resulted in lost of mitochondrial homeostasis and cell death (140). Activation of survival pathways mediated by JNK and STAT1, results in an increase of Mcl-1 in monocyte-derived macrophages and tissue macrophages (143). Mycobacterium infection of THP-1 cells induces transcriptional downregulation of Bak and Bax but upregulation of Mcl-1 (144). Bad is regulated by AKT-dependent phosphorylation of Ser^{112} and Ser^{136}, inhibition of PI3K/PDK1/AKT signal pathway, results in its translocation to mitochondria promoting apoptosis (145). In addition, dimerization of the Bcl-2-members is other important regulatory mechanism. The direct interaction of homo- and hetero-dimers results in pore formation allowing the release of apoptogenic factors to the cytoplasm (146). In monocytes, Bax/Bak and Bim/Bcl-2 associate with the VDAC1 (Voltage Dependent Activation Channel 1) in the outer mitochondrial membrane contributing to activation of apoptosis by facilitating pore formation (147, 148). Bid is the only member of the family, whose activity is regulated by cleavage. Monocytic

cells treated with TRAIL show caspase-8 activation leading to the cleavage of Bid (tBid). tBid translocation to the mitochondria, promotes the oligomerization of Bax/Bak helping to induce cell death (149).

Another important regulator of cell fate are the serine/threonine kinases belonging to the Protein kinases C family (150). The PKC family consists of 11 isoforms that is divided in classical (α, β, and γ), novel (δ, ε, η, and θ) and atypical (ζ, λ and τ) (151). The classical and atypical have anti-apoptotic properties, whereas the novels have been described as pro-apoptotic (150). The isoforms are differentially expressed in monocytes and macrophages, suggesting their possible role in monocyte differentiation and life span (152). Classical members of this family such as PKCα and β increase during PMA-stimulation and monocyte/macrophage induced-differentiation (153). Microarray analysis of myelomonocytic cells induce to differentiate with TPA show an increase in PKCα and a decrease in PKCδ expression (65). PKCε is highly activated during tumorigenesis and can also be activated in LPS-stimulated macrophages (154). Some the PKC isoforms: PKCδ, ε, and ζ are cleaved by caspase-3 suggesting the existence of a feedback loop that may regulate the activity of these enzymes (155-157). Tyrosine phosphorylation of PKCδ correlates with its translocation to the nucleus followed by apoptosis (158). Consistently, treatment of cells with the caspase-3 inhibitor DEVD-FMK, show a decrease in its cleavage and apoptotic activity (159). Our group recently found that PKCδ associates and phosphorylates caspase-3 (Figure 2). The phosphorylation of caspase-3 increases its apoptotic activity (160). This effect is specific of PKCδ, as other isoforms failed to phosphorylate caspase-3 (160). It will be interesting to know how the phosphorylation affects the ability of caspase-3 to interact with other pro- and anti-apoptotic mediators.

5.2. Anti-apoptotic regulators

Monocytes evade the apoptotic fate during inflammation, differentiation and malignant transformation. This is achieved by the contribution of survival pathways that in turn block the apoptotic cascade. While the details of this crosstalk are far from being understood, it has become clear that the inhibition of apoptosis acts at multiple levels. Monocyte/macrophage prolonged survival is mediated by the increased expression of anti-apoptotic proteins like IAPs, Bcl-2 family members, and heat shock proteins. In addition, inflammatory cytokines and chemokines stimulate monocyte migration and promote their accumulation and differentiation. Thus, chronic inflammatory diseases and cancer are characterized by an increase in monocyte/macrophage populations (161).

IAPs or "inhibitor of apoptosis" constitute a conserved family first characterized in baculovirus (162). IAPs have a common zinc-binding domain named Baculovirus IAP Repeat (BIR) (162). Eight human orthologues have been described so far including: XIAP (ILP-1, MIHA), ILP-2 (Ts-IAP), cIAP-1 (HIAP2, MIHB), cIAP-2 (HIAP1, MIHC), ML-IAP (Livin, KIAP), NAIP, Survivin (TIAP) and Apollon (Bruce). Some of the IAPs contain a second zinc-binding motif named RING that binds to the caspase-recruitment domains (CARD) (163-165). The X-linked IAP (XIAP) is the best-characterized member of this family. XIAP binds directly to caspase-3 inhibiting its activation (166). During Fas-induced apoptosis, XIAP is cleaved in two fragments, one containing two BIR domains (BIR-1 and -2) and the other containing the RING domain. BIR1-2 and the RING bind differentially to the caspases. Immunoprecipitations using recombinant proteins XIAP show that BIR 1-2 binds to caspase-3 and -7, whereas the RING motif is able to bind to caspase-9 (167). Moreover, in a cell free system approach using recombinant proteins and THP-1 cell lyses shown that, XIAP associates with caspase-9 halting the activation of the apoptosome-dependent activation of caspase-3 (164). In monocytes and myelomonocytic leukemia cells, four members of this family have been described: XIAP, c-IAP1, c-IAP2, Survivin (168, 169). Survivin has been found highly expressed in circulation and in synovial fluids of RA patients. Overexpression of recombinant survivin in monocytes has been associated with an increase on ICAM-1 expression (170). These results can suggest that survivin is related with the physiopathology of bone destruction mediated by monocyte/macrophage in RA (170). LPS or PMA stimulated monocyte-derived macrophages, show downregulation of cIAP2 mRNA correlating with an increase in the caspase-3 activity (169). XIAP is also highly expressed during monocyte/differentiation differentiation and malignant myelopoiesis, and has been proposed as a marker of poor prognosis in patients with acute myeloid leukemia (AML), suggesting that uncontrolled survival mechanisms may impair AML therapies (171).

Heat-shock proteins (Hsps) especially Hsp27, Hsp70, and Hsp90 have been implicated in defense mechanisms against apoptosis (172). Hsp70 has a protective effect during heat shock in myelomonocytic cells (172). In addition, an increase in the expression of Hsp70 has been observed during monocyte to dendritic cell differentiation (173). Increase expression of Hsp27 has been correlated with survival in response to stress and cytotoxic stimuli (174). *Ex-vivo* experiments showed that Hsp27 expression interferes with the accumulation of cytosolic cytochrome c in cancer cells (172). Hsp27 can bind to Bcl-2 family members and its expression markedly decreases Bax activation (175). Hsp27 sequestration of cytochrome c inhibits the activation of caspase-9 (176). In addition, we showed recently that Hsp27, in sharp contrast with Hsp70 is constitutively expressed in monocytes. Moreover, αβ-crystalline, the closest Hsp27 homologue is not present in primary human monocytes (69). Interestingly we found that Hsp27 interacts with the prodomain of caspase-3 inhibiting its proteolytic activation (69). Whether this inhibitory function of Hsp27 is conserved in other executioner caspases will need to be determined. Interestingly, we found that during monocyte/macrophage differentiation the level of Hsp27 increases dramatically. Consistent with its role as an anti-apoptotic regulator, silencing of Hsp27 increases the number of apoptotic macrophages (69). Thus, it will be interesting to see whether Hsp27 contributes to monocyte/macrophage differentiation.

6. MONOCYTES AND MACROPHAGES IN INFLAMMATION

The patrolling behavior of monocytes and tissue macrophages is essential in the initial host response to infection. The initiation and resolution of acute and chronic inflammation are mediated by the activation of monocytes and macrophages, which are triggered by the recognition and phagocytosis of pathogen through specialized receptors (84, 177). Active macrophages surrounding tissue damage or malignant tissues produce inflammatory cytokines that diffuse toward veins, which increase the level of inflammatory cytokines in the monocyte microenvironment (32). Monocytes become activated migrating to the tissues where they participate in tissue damage or repair and remodeling depending on the cytokines available at sites of inflammation (Figure 1). The activation of monocyte and macrophages is mediated by specialized receptors, such as MHC (see section 4.2) and the Toll-like receptor (TLR) family (84). The highly conserved TLR family is composed of nine members involved in pathogen recognition (178). TLRs are type I integral membrane glycoproteins, containing an extracellular domain with leucine-rich-repeat (LRR) motifs and a cytoplasmic domain homologous to IL-1R (TIR) (179). TLRs can be found in the cellular membrane, like in the case of TLR1, TLR2, TLR4, TLR5, and TLR6 whereas TLR3, TLR7, TLR8, and TLR9 are localized in intracellular membranes (180). Activation of TLRs triggers two signaling pathways, one mediated by the myeloid differentiation factor 88 (MyD88) and the other mediated by interferon regulatory factor 3 (IRF3) (179). TLR2, TLR4, TLR7, TLR9 activate the MyD88 multi-step signaling pathway, resulting in activation and translocation of NF-κβ and IRF3 to the nucleus promoting the production of cytokines, IL-1β and TNFα (Figure 2) (84). TLR3 activates IRF3 signal pathway, resulting in the activation and translocation of IRF3 to the nucleus promoting the production of IL-1β (181). In monocytes and macrophages TLR1, TLR2, TLR4, TLR5 and TLR6 have been identified in the plasma membrane (182). Macrophages express the TLR3, TLR7, and TLR9 intracellulary the endosome membranes, whereas only TLR9 is found in the endosome membranes in monocytes (8, 181, 182). LPS purified form $Gram^-$ bacteria and lipopeptide (BLP) from $Gram^+$ have been excellent tools to study the processes of monocyte/macrophage activation (183). For example, the signal transduction pathway activated by these pathogens was studied in bone marrow macrophages (BMM) derived form the MyD88 knockout mice. Microarray analysis of BMM treated with the $Gram^+$ *L. Monocytogenes* show that MyD88-/- macrophages fail to produce inflammatory cytokines such as IL-1β, IL-6 and TNFα and also NO (184). The activation of the IRF3 signaling pathway was evaluated in RAW macrophages treated with poly (I:C), a synthetic TLR3 ligand. These experiments showed the activation NF-κβ and the increased production of IL-1β (181). *Ex-vivo* experiments using BMM obtained from TLR2+/+TLR4-/- or TLR2-/-TLR4+/+ mice treated with the *M. Lipomannan, a* $Gram^-$ bacteria, demonstrated that TNFα and NO production are mediated by TLR4 (185). These findings are in agreement with results obtained using purified BLP or LPS in the same animals (185). Together these data show that TNFα production is TLR-dependent, with BLP activating the pathway via TLR2 and LPS via TLR4 (185). As we previously described (section 3.1 Subpopulation of Monocytes), different subpopulations of monocytes are able to induce inflammatory cytokines upon LPS stimulation. Notably, $CD14^{high}CD16^+$ are the main producers of IL-10, whereas $CD14^{dim}CD16^+$ produce TNFα (28). Comparative studies in monocytes and monocyte-derived macrophages showed that TLR agonists induce similar levels of inflammatory cytokines except in the case of poly (I:C), which induces a major induction of TNFα in macrophages (182). TAMs can also recognize pathogens trough the TLR contributing to the inflammatory milieu of the tumor (186).

The resolution of inflammation is initiated when the stimuli has been eliminated, resulting in the downregulation of inflammatory cytokines and the upregulation of prostaglandin E_2 (PGE_2) (187) and leukotrienes (LTs). PGE_2 helps decreasing the exudation and blood flow into the inflammatory tissue (188). Administration of LTs in the local exudates of inflammation results in a decrease of TNFα (189). In addition, reactivation of the apoptotic machinery contributes to tissue repair and to the clearance of activated monocytes and other leukocytes (190). The resolution process can be short or acute taking just few days. Otherwise, it can be longer up to years constituting a chronic condition. The mechanisms causing the persistence of inflammation are not yet fully understood but it is clear that high levels of inflammatory cytokines in circulation are key elements in chronic inflammation in RA and chronic colitis (43, 191).

Based on these findings therapeutic approaches to target inflammation has been based on the ability to reduce inflammatory cytokines. In this context, current use of anti-TNFα as an anti-inflammatory therapy is used in patients with chronic inflammation that has negative results with the conventional therapies (191). TNFα and IL-6, IL8 and IL-10 inhibitors have been used as alternative therapies in patients with inflammatory conditions, such as RA and chronic colitis (192-194). However, these studies have reported only 50-60% success (191, 194). In line with these results it could be of great significance to define new additional therapeutic approaches targeting activated monocytes to undergo apoptosis. This approach could help reducing inflammatory cytokines at the same time that contributes to the clearance of activated monocytes at sites of inflammation.

7. CONCLUSIONS

Monocytes and macrophages act as key guardians mediating innate immunity and participating in the acquired immunity. Moreover, different subpopulations of monocytes have being recently characterized and are being recognized to have a very different contribution to innate immunity and differentiation. Monocytes and macrophages originate from a shared progenitor, express similar receptors, and activate a comparable cascade of

inflammatory mediators. However, they differ dramatically in their cellular life span. Monocytes can switch from being short live undergoing apoptosis in a day to presenting a prolonged survival during inflammation and quickly then to a short live as the inflammation resolves. Macrophages' life span has less plasticity they live longer and are quite resistant to apoptotic stimuli. While much is known about the apoptotic and survival pathways it is noteworthy how little is known about the cross-talk of this complex network and its unique regulation in different lineages. More understanding of these regulatory pathways will potentially allow control inflammation by us to manipulating monocyte/macrophage lifespan.

8. ACKNOWLEDGMENTS

Work in Dr. Doseff's lab is supported by grants NIH (R01 HL075040-01) and NSF-MCB (0542244). We apologize to our colleagues whom made important contributions but were omitted due to space limitation. We would like to thank Dr. T. Eubank for his help with the illustrations.

9. REFERENCES

1. Shields J: Mononuclear cells, hyaline bodies and the plasma: an analytic review. *Blood* 17, 235-251 (1961)

2. Grove J. E, E. Bruscia, D. S. Krause: Plasticity of bone marrow-derived stem cells. *Stem Cells* 22, 487-500 (2004)

3. Weisbart R. H, J. C. Gasson, D. W. Golde: Colony-stimulating factors and host defense. *Ann Intern Med* 110, 297-303 (1989)

4. Tushinski R. J, I. T. Oliver, L. J. Guilbert, P. W. Tynan, J. R. Warner, E. R. Stanley: Survival of mononuclear phagocytes depends on a lineage-specific growth factor that the differentiated cells selectively destroy. *Cell* 28, 71-81 (1982)

5. van Furth R, Z. A. Cohn, J. G. Hirsch, J. H. Humphrey, W. G. Spector, H. L. Langevoort: The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. *Bull World Health Organ* 46, 845-852 (1972)

6. van Furth R, Z. A. Cohn: The origin and kinetics of mononuclear phagocytes. *J Exp Med* 128, 415-435 (1968)

7. Fahy R. J, A. I. Doseff, M. D. Wewers: Spontaneous human monocyte apoptosis utilizes a caspase-3-dependent pathway that is blocked by endotoxin and is independent of caspase-1. *J Immunol* 163, 1755-1762 (1999)

8. O'Mahony D. S, U. Pham, R. Iyer, T. R. Hawn, W. C. Liles: Differential constitutive and cytokine-modulated expression of human Toll-like receptors in primary neutrophils, monocytes, and macrophages. *Int J Med Sci* 5, 1-8 (2008)

9. Auffray C, D. Fogg, M. Garfa, G. Elain, O. Join-Lambert, S. Kayal, S. Sarnacki, A. Cumano, G. Lauvau, F. Geissmann: Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science* 317, 666-670 (2007)

10. Geissmann F, S. Jung, D. R. Littman: Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* 19, 71-82 (2003)

11. van Furth R: Human monocytes and cytokines. *Res Immunol* 149, 719-720 (1998)

12. Ancuta P, R. Rao, A. Moses, A. Mehle, S. K. Shaw, F. W. Luscinskas, D. Gabuzda: Fractalkine preferentially mediates arrest and migration of $CD16^+$ monocytes. *J Exp Med* 197, 1701-1707 (2003)

13. Charo I. F, M. B. Taubman: Chemokines in the pathogenesis of vascular disease. *Circ Res* 95, 858-866 (2004)

14. Mangan D. F, S. M. Wahl: Differential regulation of human monocyte programmed cell death (apoptosis) by chemotactic factors and pro-inflammatory cytokines. *J Immunol* 147, 3408-3412 (1991)

15. Goyal A, Y. Wang, M. M. Graham, A. I. Doseff, N. Y. Bhatt, C. B. Marsh: Monocyte survival factors induce Akt activation and suppress caspase-3. *Am J Respir Cell Mol Biol* 26, 224-230 (2002)

16. Bianchi G, F. Montecucco, M. Bertolotto, F. Dallegri, L. Ottonello: Immune complexes induce monocyte survival through defined intracellular pathways. *Ann N Y Acad Sci* 1095, 209-219 (2007)

17. Blom A. B, T. R. Radstake, A. E. Holthuysen, A. W. Sloetjes, G. J. Pesman, F. G. Sweep, F. A. van de Loo, L. A. Joosten, P. Barrera, P. L. van Lent, W. B. van den Berg: Increased expression of Fcγ receptors II and III on macrophages of rheumatoid arthritis patients results in higher production of tumor necrosis factor alpha and matrix metalloproteinase. *Arthritis Rheum* 48, 1002-1014 (2003)

18. van Furth R, W. Sluiter: Distribution of blood monocytes between a marginating and a circulating pool. *J Exp Med* 163, 474-479 (1986)

19. Goyert S. M, E. Ferrero, W. J. Rettig, A. K. Yenamandra, F. Obata, M. M. Le Beau: The CD14 monocyte differentiation
antigen maps to a region encoding growth factors and receptors. *Science* 239, 497-500 (1988)

20. Passlick B, D. Flieger, H. W. Ziegler-Heitbrock: Identification and characterization of a novel monocyte subpopulation in human peripheral blood. *Blood* 74, 2527-2534 (1989)

21. O'Doherty U, M. Peng, S. Gezelter, W. J. Swiggard, M. Betjes, N. Bhardwaj, R. M. Steinman: Human blood contains two subsets of dendritic cells, one immunologically mature and the other immature. *Immunology* 82, 487-493 (1994)

22. Frankenberger M, T. Sternsdorf, H. Pechumer, A. Pforte, H. W. Ziegler-Heitbrock: Differential cytokine expression in human blood monocyte subpopulations: a polymerase chain reaction analysis. *Blood* 87, 373-377 (1996)

23. Mizuno K, T. Toma, H. Tsukiji, H. Okamoto, H. Yamazaki, K. Ohta, K. Ohta, Y. Kasahara, S. Koizumi, A. Yachie: Selective expansion of $CD16^{high}CCR2^{-}$ subpopulation of circulating monocytes with preferential production of haem oxygenase (HO)-1 in response to acute inflammation. *Clin Exp Immunol* 142, 461-470 (2005)

24. Skrzeczynska-Moncznik J, M. Bzowska, S. Loseke, E. Grage-Griebenow, M. Zembala, J. Pryjma: Peripheral blood $CD14^{high}$ $CD16^{+}$ monocytes are main producers of IL-10. *Scand J Immunol* 67, 152-159 (2008)

25. Fingerle G, A. Pforte, B. Passlick, M. Blumenstein, M. Strobel, H. W. Ziegler-Heitbrock: The novel subset of $CD14^{+}CD16^{+}$ blood monocytes is expanded in sepsis patients. *Blood* 82, 3170-3176 (1993)

26. Kruger M, J. G. Van de Winkel, T. P. De Wit, L. Coorevits, J. L. Ceuppens: Granulocyte-macrophage colony-stimulating factor down-regulates CD14 expression on monocytes. *Immunology* 89, 89-95 (1996)

27. Wu H, K. Moulton, A. Horvai, S. Parik, C. K. Glass: Combinatorial interactions between AP-1 and ets domain proteins contribute to the developmental regulation of the macrophage scavenger receptor gene. *Mol Cell Biol* 14, 2129-2139 (1994)

28. Kanai T, S. Makita, T. Kawamura, Y. Nemoto, D. Kubota, K. Nagayama, T. Totsuka, M. Watanabe: Extracorporeal elimination of TNFα-producing $CD14^{dull}CD16^{+}$ monocytes in leukocytapheresis therapy for ulcerative colitis. *Inflamm Bowel Dis* 13, 284-290 (2007)

29. Yoshioka Y, A. Ohwada, N. Harada, N. Satoh, S. Sakuraba, T. Dambara, Y. Fukuchi: Increased circulating $CD14^{dim}CD16^{+}$ monocytes in a patient with pulmonary alveolar proteinosis. *Respirology* 7, 273-279 (2002)

30. Skinner N. A, C. M. MacIsaac, J. A. Hamilton, K. Visvanathan: Regulation of Toll-like receptor TLR2 and TLR4 on $CD14^{dim}CD16^{+}$ monocytes in response to sepsis-related antigens. *Clin Exp Immunol* 141, 270-278 (2005)

31. Ulrich C, G. H. Heine, M. K. Gerhart, H. Kohler, M. Girndt: Proinflammatory $CD14^{+}CD16^{+}$ monocytes are associated with subclinical atherosclerosis in renal transplant patients. *Am J Transplant* 8, 103-110 (2008)

32. Schober A, C. Weber: Mechanisms of monocyte recruitment in vascular repair after injury. *Antioxid Redox Signal* 7, 1249-1257 (2005)

33. Beekhuizen H, A. J. Corsel-Van Tilburg, I. Blokland, R. Van Furth: Characterization of the adherence of human monocytes to cytokine-stimulated human macrovascular endothelial cells. *Immunology* 74, 661-669 (1991)

34. Beekhuizen H, E. M. Verdegaal, I. Blokland, R. van Furth: Contribution of ICAM-1 and VCAM-1 to the morphological changes in monocytes bound to human venous endothelial cells stimulated with recombinant interleukin-4 (rIL-4) or rIL-1α. *Immunology* 77, 469-472 (1992)

35. Beekhuizen H, R. Van Furth: Monocyte adherence to human vascular endothelium. *Behring Inst Mitt* 63-86 (1993)

36. Xu H, A. Manivannan, R. Dawson, I. J. Crane, M. Mack, P. Sharp, J. Liversidge: Differentiation to the $CCR2^{+}$ inflammatory phenotype in vivo is a constitutive, time-limited property of blood monocytes and is independent of local inflammatory mediators. *J Immunol* 175, 6915-6923 (2005)

37. Charo I. F, S. J. Myers, A. Herman, C. Franci, A. J. Connolly, S. R. Coughlin: Molecular cloning and functional expression of two monocyte chemoattractant protein 1 receptors reveals alternative splicing of the carboxyl-terminal tails. *Proc Natl Acad Sci U S A* 91, 2752-2756 (1994)

38. Jung S, J. Aliberti, P. Graemmel, M. J. Sunshine, G. W. Kreutzberg, A. Sher, D. R. Littman: Analysis of fractalkine receptor CX_3CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Mol Cell Biol* 20, 4106-4114 (2000)

39. Schulz C, A. Schafer, M. Stolla, S. Kerstan, M. Lorenz, M. L. von Bruhl, M. Schiemann, J. Bauersachs, T. Gloe, D. H. Busch, M. Gawaz, S. Massberg: Chemokine fractalkine mediates leukocyte recruitment to inflammatory endothelial cells in flowing whole blood: a critical role for P-selectin expressed on activated platelets. *Circulation* 116, 764-773 (2007)

40. Ziegler-Heitbrock H. W, G. Fingerle, M. Strobel, W. Schraut, F. Stelter, C. Schutt, B. Passlick, A. Pforte: The novel subset of $CD14^{+}/CD16^{+}$ blood monocytes exhibits features of tissue macrophages. *Eur J Immunol* 23, 2053-2058 (1993)

41. Nasu N, S. Yoshida, S. Akizuki, Y. Higuchi, M. Setoguchi, S. Yamamoto: Molecular and physiological properties of murine CD14. *Int Immunol* 3, 205-213 (1991)

42. Fleming T. J, M. L. Fleming, T. R. Malek: Selective expression of Ly-6G on myeloid lineage cells in mouse bone marrow. RB6-8C5 mAb to granulocyte-differentiation antigen (Gr-1) detects members of the Ly-6 family. *J Immunol* 151, 2399-2408 (1993)

43. Tacke F, D. Alvarez, T. J. Kaplan, C. Jakubzick, R. Spanbroek, J. Llodra, A. Garin, J. Liu, M. Mack, N. van Rooijen, S. A. Lira, A. J. Habenicht, G. J. Randolph: Monocyte subsets differentially employ CCR2, CCR5, and CX_3CR1 to accumulate within atherosclerotic plaques. *J Clin Invest* 117, 185-194 (2007)

44. Aschoff L: Das reticulo-endotheliale system. *Ergeb Inn Med Kinderheilkd* 26, 1-118 (1924)

45. Laskin D. L, B. Weinberger, J. D. Laskin: Functional heterogeneity in liver and lung macrophages. *J Leukoc Biol* 70, 163-170 (2001)

46. Guillemin G. J, B. J. Brew: Microglia, macrophages, perivascular macrophages, and pericytes: a review of function and identification. *J Leukoc Biol* 75, 388-397 (2004)

47. Stout R. D, C. Jiang, B. Matta, I. Tietzel, S. K. Watkins, J. Suttles: Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. *J Immunol* 175, 342-349 (2005)

48. Roos J, M. Auger: The biology of the macrophage. In: The macrophage. Eds: Burke B, Lewis C, US. 1-72 (2002)

49. Cecchini M. G, W. Hofstetter, J. Halasy, A. Wetterwald, R. Felix: Role of CSF-1 in bone and bone marrow development. *Mol Reprod Dev* 46, 75-84 (1997)

50. Stanley E, G. J. Lieschke, D. Grail, D. Metcalf, G. Hodgson, J. A. Gall, D. W. Maher, J. Cebon, V. Sinickas, A. R. Dunn: Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology. *Proc Natl Acad Sci U S A* 91, 5592-5596 (1994)

51. Wiktor-Jedrzejczak W, A. Bartocci, A. W. Ferrante, Jr., A. Ahmed-Ansari, K. W. Sell, J. W. Pollard, E. R. Stanley: Total absence of colony-stimulating factor 1 in the macrophage-deficient osteopetrotic (*op/op*) mouse. *Proc Natl Acad Sci U S A* 87, 4828-4832 (1990)

52. Begg S. K, J. M. Radley, J. W. Pollard, O. T. Chisholm, E. R. Stanley, I. Bertoncello: Delayed hematopoietic development in osteopetrotic (op/op) mice. *J Exp Med* 177, 237-242 (1993)

53. Glasow A, N. Prodromou, K. Xu, M. von Lindern, A. Zelent: Retinoids and myelomonocytic growth factors cooperatively activate RARA and induce human myeloid leukemia cell differentiation via MAP kinase pathways. *Blood* 105, 341-349 (2005)

54. Kelley T. W, M. M. Graham, A. I. Doseff, R. W. Pomerantz, S. M. Lau, M. C. Ostrowski, T. F. Franke, C. B. Marsh: Macrophage colony-stimulating factor promotes cell survival through Akt/protein kinase B. *J Biol Chem* 274, 26393-26398 (1999)

55. van der Meer J. W, J. van de Gevel, R. van Furth: In vitro proliferation of mononuclear phagocytes from murine and human bone marrow. *Immunobiology* 161, 212-218 (1982)

56. van der Meer J. W, J. S. van de Gevel, R. H. Beelen, D. Fluitsma, E. C. Hoefsmit, R. van Furth: Culture of human bone marrow in the teflon culture bag: identification of the human monoblast. *J Reticuloendothel Soc* 32, 355-369 (1982)

57. van der Meer J. W, J. S. van de Gevel, A. Blusse van Oud Alblas, J. A. Kramps, T. L. van Zwet, P. C. Leijh, R. van Furth: Characteristics of human monocytes cultured in the Teflon culture bag. *Immunology* 47, 617-625 (1982)

58. Fischer H. G, S. Frosch, K. Reske, A. B. Reske-Kunz: Granulocyte-macrophage colony-stimulating factor activates macrophages derived from bone marrow cultures to synthesis of MHC class II molecules and to augmented antigen presentation function. *J Immunol* 141, 3882-3888 (1988)

59. Longoni D, L. Piemonti, S. Bernasconi, A. Mantovani P. Allavena: Interleukin-10 increases mannose receptor expression and endocytic activity in monocyte-derived dendritic cells. *Int J Clin Lab Res* 28, 162-169 (1998)

60. Morishima S, I. Morita, T. Tokushima, H. Kawashima, M. Miyasaka, K. Omura, S. Murota: Expression and role of mannose receptor/terminal high-mannose type oligosaccharide on osteoclast precursors during osteoclast formation. *J Endocrinol* 176, 285-292 (2003)

61. Taylor P. R, L. Martinez-Pomares, M. Stacey, H. H. Lin, G. D. Brown, S. Gordon: Macrophage receptors and immune recognition. *Annu Rev Immunol* 23, 901-944 (2005)

62. Blum J. S, P. D. Stahl, R. Diaz, M. L. Fiani: Purification and characterization of the D-mannose receptor from J774 mouse macrophage cells. *Carbohydr Res* 213, 145-153 (1991)

63. Lendemans S, M. Rani, C. Selbach, E. Kreuzfelder, F. U. Schade, S. Flohe: GM-CSF priming of human monocytes is dependent on ERK1/2 activation. *J Endotoxin Res* 12, 10-20 (2006)

64. Hashimoto S, T. Suzuki, H. Y. Dong, N. Yamazaki, K. Matsushima: Serial analysis of gene expression in human monocytes and macrophages. *Blood* 94, 837-844 (1999)

65. Juan H. F, J. Y. Lin, W. H. Chang, C. Y. Wu, T. L. Pan, M. J. Tseng, K. H. Khoo, S. T. Chen: Biomic study of human myeloid leukemia cells differentiation to macrophages using DNA array, proteomic, and bioinformatic analytical methods. *Electrophoresis* 23, 2490-2504 (2002)

66. Kohro T, T. Tanaka, T. Murakami, Y. Wada, H. Aburatani, T. Hamakubo, T. Kodama: A comparison of

differences in the gene expression profiles of phorbol 12-myristate 13-acetate differentiated THP-1 cells and human monocyte-derived macrophage. *J Atheroscler Thromb* 11, 88-97 (2004)

67. Liu H, B. Shi, C. C. Huang, P. Eksarko, R. M. Pope: Transcriptional diversity during monocyte to macrophage differentiation. *Immunol Lett* 117, 70-80 (2008)

68. Scott E. W, M. C. Simon, J. Anastasi, H. Singh: Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. *Science* 265, 1573-1577 (1994)

69. Voss O. H, S. Batra, S. J. Kolattukudy, M. E. Gonzalez-Mejia, J. B. Smith, A. I. Doseff: Binding of caspase-3 prodomain to heat shock protein 27 regulates monocyte apoptosis by inhibiting caspase-3 proteolytic activation. *J Biol Chem* 282, 25088-25099 (2007)

70. Jin M, J. M. Opalek, C. B. Marsh, H. M. Wu: Proteome comparison of alveolar macrophages with monocytes reveals distinct protein characteristics. *Am J Respir Cell Mol Biol* 31, 322-329 (2004)

71. Lewis C. E, J. W. Pollard: Distinct role of macrophages in different tumor microenvironments. *Cancer Res* 66, 605-612 (2006)

72. Chen L, S. N. Willis, A. Wei, B. J. Smith, J. I. Fletcher, M. G. Hinds, P. M. Colman, C. L. Day, J. M. Adams, D. C. Huang: Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. *Mol Cell* 17, 393-403 (2005)

73. Matsumoto E, M. Hatanaka, M. Bohgaki, S. Maeda: PKC pathway and ERK/MAPK pathway are required for induction of cyclin D1 and $p21^{Waf1}$ during 12-o-tetradecanoylphorbol 13-acetate-induced differentiation of myeloleukemia cells. *Kobe J Med Sci* 52, 181-194 (2006)

74. Asada M, T. Yamada, K. Fukumuro, S. Mizutani: p21Cip1/WAF1 is important for differentiation and survival of U937 cells. *Leukemia* 12, 1944-1950 (1998)

75. Brugarolas J, C. Chandrasekaran, J. I. Gordon, D. Beach, T. Jacks, G. J. Hannon: Radiation-induced cell cycle arrest compromised by p21 deficiency. *Nature* 377, 552-557 (1995)

76. Scatizzi J. C, J. Hutcheson, E. Bickel, J. M. Woods, K. Klosowska, T. L. Moore, G. K. Haines, 3rd, H. Perlman: $p21^{Cip1}$ is required for the development of monocytes and their response to serum transfer-induced arthritis. *Am J Pathol* 168, 1531-1541 (2006)

77. Leibovich S. J, R. Ross: The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum. *Am J Pathol* 78, 71-100 (1975)

78. Fadok V. A, D. L. Bratton, A. Konowal, P. W. Freed, J. Y. Westcott, P. M. Henson: Macrophages that have ingested apoptotic cells *in vitro* inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-β, PGE_2, and PAF. *J Clin Invest* 101, 890-898 (1998)

79. Trautmann A, S. Valitutti: The diversity of immunological synapses. *Current Opinion in Immunology* 15, 249-254 (2003)

80. Shackelford D. A, J. F. Kaufman, A. J. Korman, J. L. Strominger: HLA-DR antigens: structure, separation of subpopulations, gene cloning and function. *Immunol Rev* 66, 133-187 (1982)

81. Cresswell P: Assembly, transport, and function of MHC class II molecules. *Annu Rev Immunol* 12, 259-293 (1994)

82. Santin A. D, P. L. Hermonat, A. Ravaggi, M. Chiriva-Internati, M. J. Cannon, J. C. Hiserodt, S. Pecorelli, G. P. Parham: Expression of surface antigens during the differentiation of human dendritic cells vs macrophages from blood monocytes in vitro. *Immunobiology* 200, 187-204 (1999)

83. Waldburger J. M, T. Suter, A. Fontana, H. Acha-Orbea, W. Reith: Selective abrogation of major histocompatibility complex class II expression on extrahematopoietic cells in mice lacking promoter IV of the class II transactivator gene. *J Exp Med* 194, 393-406 (2001)

84. Akira S, S. Uematsu, O. Takeuchi: Pathogen recognition and innate immunity. *Cell* 124, 783-801 (2006)

85. de Almeida C. J, R. Linden: Phagocytosis of apoptotic cells: a matter of balance. *Cell Mol Life Sci* 62, 1532-1546 (2005)

86. Callahan M. K, P. Williamson, R. A. Schlegel: Surface expression of phosphatidylserine on macrophages is required for phagocytosis of apoptotic thymocytes. *Cell Death Differ* 7, 645-653 (2000)

87. Arur S, U. E. Uche, K. Rezaul, M. Fong, V. Scranton, A. E. Cowan, W. Mohler, D. K. Han: Annexin I is an endogenous ligand that mediates apoptotic cell engulfment. *Dev Cell* 4, 587-598 (2003)

88. Fadok V. A, D. L. Bratton, S. C. Frasch, M. L. Warner, P. M. Henson: The role of phosphatidylserine in recognition of apoptotic cells by phagocytes. *Cell Death Differ* 5, 551-562 (1998)

89. Platt N, H. Suzuki, Y. Kurihara, T. Kodama, S. Gordon: Role for the class A macrophage scavenger receptor in the phagocytosis of apoptotic thymocytes in vitro. *Proc Natl Acad Sci U S A* 93, 12456-12460 (1996)

90. Luciani M. F, G. Chimini: The ATP binding cassette transporter ABC1, is required for the engulfment of corpses generated by apoptotic cell death. *Embo J* 15, 226-235 (1996)

91. Underhill D. M, B. Gantner: Integration of Toll-like receptor and phagocytic signaling for tailored immunity. *Microbes Infect* 6, 1368-1373 (2004)

92. Underhill D. M, A. Ozinsky: Toll-like receptors: key mediators of microbe detection. *Curr Opin Immunol* 14, 103-110 (2002)

93. Underhill D. M, A. Ozinsky: Phagocytosis of microbes: complexity in action. *Annu Rev Immunol* 20, 825-852 (2002)

94. Duvall E, A. H. Wyllie, R. G. Morris: Macrophage recognition of cells undergoing programmed cell death (apoptosis). *Immunology* 56, 351-358 (1985)

95. Savill J, I. Dransfield, N. Hogg, C. Haslett: Vitronectin receptor-mediated phagocytosis of cells undergoing apoptosis. *Nature* 343, 170-173 (1990)

96. Metchnikoff E: Immunity in infective diseases. Eds: Cambridge University Press, London, Glasgow. 1-168 (1905)

97. Souza G. M, D. P. Mehta, M. Lammertz, J. Rodriguez-Paris, R. Wu, J. A. Cardelli, H. H. Freeze: Dictyostelium lysosomal proteins with different sugar modifications sort to functionally distinct compartments. *J Cell Sci* 110 (Pt 18), 2239-2248 (1997)

98. Melo R. C, D. L. Fabrino, H. D'Avila, H. C. Teixeira, A. P. Ferreira: Production of hydrogen peroxide by peripheral blood monocytes and specific macrophages during experimental infection with *Trypanosoma cruzi in vivo*. *Cell Biol Int* 27, 853-861 (2003)

99. Jaramillo M, M. Olivier: Hydrogen peroxide induces murine macrophage chemokine gene transcription via extracellular signal-regulated kinase- and cyclic adenosine 5'-monophosphate (cAMP)-dependent pathways: involvement of NF-κB, activator protein 1, and cAMP response element binding protein. *J Immunol* 169, 7026-7038 (2002)

100. Tilney L. G, D. A. Portnoy: Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, *Listeria monocytogenes*. *J Cell Biol* 109, 1597-1608 (1989)

101. Waghabi M. C, C. M. Coutinho, M. N. Soeiro, M. C. Pereira, J. J. Feige, M. Keramidas, A. Cosson, P. Minoprio, F. Van Leuven, T. C. Araujo-Jorge: Increased *Trypanosoma cruzi* invasion and heart fibrosis associated with high transforming growth factor β levels in mice deficient in α(2)-macroglobulin. *Infect Immun* 70, 5115-5123 (2002)

102. Perez-Fuentes R., A. Lopez-Colombo, G. Ordonez-Toquero, I. Gomez-Albino, J. Ramos, E. Torres-Rasgado, H. Salgado-Rosas, M. Romero-Diaz, P. Pulido-Perez, M. C. Sanchez-Guillen: Correlation of the serum concentrations of tumour necrosis factor and nitric oxide with disease severity in chronic Chagas disease (American trypanosomiasis). *Ann Trop Med Parasitol* 101, 123-132 (2007)

103. Burnett S. H, B. J. Beus, R. Avdiushko, J. Qualls, A. M. Kaplan, D. A. Cohen: Development of peritoneal adhesions in macrophage depleted mice. *J Surg Res* 131, 296-301 (2006)

104. Burnett S. H, E. J. Kershen, J. Zhang, L. Zeng, S. C. Straley, A. M. Kaplan, D. A. Cohen: Conditional macrophage ablation in transgenic mice expressing a Fas-based suicide gene. *J Leukoc Biol* 75, 612-623 (2004)

105. Doseff A. I: Apoptosis: the sculptor of development. *Stem Cells Dev* 13, 473-483 (2004)

106. White E: Life, death, and the pursuit of apoptosis. *Genes Dev* 10, 1-15 (1996)

107. Thornberry N. A, Y. Lazebnik: Caspases: enemies within. *Science* 281, 1312-1316 (1998)

108. Creagh E. M, H. Conroy, S. J. Martin: Caspase-activation pathways in apoptosis and immunity. *Immunol Rev* 193, 10-21 (2003)

109. Fan T. J, L. H. Han, R. S. Cong, J. Liang: Caspase family proteases and apoptosis. *Acta Biochim Biophys Sin* 37, 719-727 (2005)

110. Sansonetti P. J, A. Phalipon, J. Arondel, K. Thirumalai, S. Banerjee, S. Akira, K. Takeda, A. Zychlinsky: Caspase-1 activation of IL-1β and IL-18 are essential for Shigella flexneri-induced inflammation. *Immunity* 12, 581-590 (2000)

111. Rowe S. J., L. Allen, V. C. Ridger, P. G. Hellewell, M. K. Whyte: Caspase-1-deficient mice have delayed neutrophil apoptosis and a prolonged inflammatory response to lipopolysaccharide-induced acute lung injury. *J Immunol* 169, 6401-6407 (2002)

112. Martinon F, J. Tschopp: Inflammatory caspases and inflammasomes: master switches of inflammation. *Cell Death Differ* 14, 10-22 (2007)

113. Alcivar A, S. Hu, J. Tang, X. Yang: DEDD and DEDD2 associate with caspase-8/10 and signal cell death. *Oncogene* 22, 291-297 (2003)

114. Ashkenazi A, V. M. Dixit: Death receptors: signaling and modulation. *Science* 281, 1305-1308 (1998)

115. Brown N. J, J. Hutcheson, E. Bickel, J. C. Scatizzi, L. D. Albee, G. K. Haines, 3rd, J. Eslick, K. Bradley, E. Taricone, H. Perlman: Fas death receptor signaling represses monocyte numbers and macrophage activation in vivo. *J Immunol* 173, 7584-7593 (2004)

116. Suliman A, A. Lam, R. Datta, R. K. Srivastava: Intracellular mechanisms of TRAIL: apoptosis through

mitochondrial-dependent and -independent pathways. *Oncogene* 20, 2122-2133 (2001)

117. Schwandner R, K. Wiegmann, K. Bernardo, D. Kreder, M. Kronke: TNF receptor death domain-associated proteins TRADD and FADD signal activation of acid sphingomyelinase. *J Biol Chem* 273, 5916-5922 (1998)

118. Green D. R, G. Kroemer: The pathophysiology of mitochondrial cell death. *Science* 305, 626-629 (2004)

119. Scaffidi C, J. P. Medema, P. H. Krammer, M. E. Peter: FLICE is predominantly expressed as two functionally active isoforms, caspase-8/a and caspase-8/b. *J Biol Chem* 272, 26953-26958 (1997)

120. Barnhart B. C, J. C. Lee, E. C. Alappat, M. E. Peter: The death effector domain protein family. *Oncogene* 22, 8634-8644 (2003)

121. Medema J. P, C. Scaffidi, F. C. Kischkel, A. Shevchenko, M. Mann, P. H. Krammer, M. E. Peter: FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). *Embo J* 16, 2794-2804 (1997)

122. Kiener P. A, P. M. Davis, G. C. Starling, C. Mehlin, S. J. Klebanoff, J. A. Ledbetter, W. C. Liles: Differential induction of apoptosis by Fas-Fas ligand interactions in human monocytes and macrophages. *J Exp Med* 185, 1511-1516 (1997)

123. Nagata S, T. Suda: Fas and Fas ligand: *lpr* and *gld* mutations. *Immunol Today* 16, 39-43 (1995)

124. Varfolomeev E. E, M. Schuchmann, V. Luria, N. Chiannilkulchai, J. S. Beckmann, I. L. Mett, D. Rebrikov, V. M. Brodianski, O. C. Kemper, O. Kollet, T. Lapidot, D. Soffer, T. Sobe, K. B. Avraham, T. Goncharov, H. Holtmann, P. Lonai, D. Wallach: Targeted disruption of the mouse caspase-8 gene ablates cell death induction by the TNF receptors, Fas/Apo1, and DR3 and is lethal prenatally. *Immunity* 9, 267-276 (1998)

125. Kuida K., T. F. Haydar, C. Y. Kuan, Y. Gu, C. Taya, H. Karasuyama, M. S. Su, P. Rakic, R. A. Flavell: Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase-9. *Cell* 94, 325-337 (1998)

126. Kuida K., T. S. Zheng, S. Na, C. Kuan, D. Yang, H. Karasuyama, P. Rakic, R. A. Flavell: Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice. *Nature* 384, 368-372 (1996)

127. Cui H, A. Schroering, H. F. Ding: p53 mediates DNA damaging drug-induced apoptosis through a caspase-9-dependent pathway in SH-SY5Y neuroblastoma cells. *Mol Cancer Ther* 1, 679-686 (2002)

128. Li P, D. Nijhawan, I. Budihardjo, S. M. Srinivasula, M. Ahmad, E. S. Alnemri, X. Wang: Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91, 479-489 (1997)

129. Cain K, D. G. Brown, C. Langlais, G. M. Cohen: Caspase activation involves the formation of the apoptosome, a large (approximately 700 kDa) caspase-activating complex. *J Biol Chem* 274, 22686-22692 (1999)

130. Gao Z, Y. Tian, J. Wang, Q. Yin, H. Wu, Y. M. Li, X. Jiang: A dimeric Smac/diablo peptide directly relieves caspase-3 inhibition by XIAP. Dynamic and cooperative regulation of XIAP by Smac/Diablo. *J Biol Chem* 282, 30718-30727 (2007)

131. Sordet O, C. Rebe, I. Leroy, J. M. Bruey, C. Garrido, C. Miguet, G. Lizard, S. Plenchette, L. Corcos, E. Solary: Mitochondria-targeting drugs arsenic trioxide and lonidamine bypass the resistance of TPA-differentiated leukemic cells to apoptosis. *Blood* 97, 3931-3940 (2001)

132. Vaux D. L, S. Cory, J. M. Adams: Bcl-2 gene promotes haemopoietic cell survival and cooperates with *c-myc* to immortalize pre-B cells. *Nature* 335, 440-442 (1988)

133. Cory S, J. M. Adams: The Bcl2 family: regulators of the cellular life-or-death switch. *Nat Rev Cancer* 2, 647-656 (2002)

134. Burlacu A: Regulation of apoptosis by Bcl-2 family proteins. *J Cell Mol Med* 7, 249-257 (2003)

135. Dasmahapatra G, J. A. Almenara, S. Grant: Flavopiridol and histone deacetylase inhibitors promote mitochondrial injury and cell death in human leukemia cells that overexpress Bcl-2. *Mol Pharmacol* 69, 288-298 (2006)

136. Dai Y, P. Dent, S. Grant: Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) promotes mitochondrial dysfunction and apoptosis induced by 7-hydroxystaurosporine and mitogen-activated protein kinase kinase inhibitors in human leukemia cells that ectopically express Bcl-2 and Bcl-xL. *Mol Pharmacol* 64, 1402-1409 (2003)

137. Berthier A, S. Lemaire-Ewing, C. Prunet, S. Monier, A. Athias, G. Bessede, J. P. Pais de Barros, A. Laubriet, P. Gambert, G. Lizard, D. Neel: Involvement of a calcium-dependent dephosphorylation of BAD associated with the localization of Trpc-1 within lipid rafts in 7-ketocholesterol-induced THP-1 cell apoptosis. *Cell Death Differ* 11, 897-905 (2004)

138. Chatterjee D, P. Pantazis, G. Li, T. A. Bremner, E. A. Hendrickson, J. H. Wyche: Susceptibility to apoptosis is restored in human leukemia HCW-2 cells following induction and stabilization of the apoptotic effector Bak. *Oncogene* 19, 4108-4116 (2000)

139. Aggarwal B. B: Nuclear factor-kappaB: the enemy within. *Cancer Cell* 6, 203-208 (2004)

140. Pagliari L. J, H. Perlman, H. Liu, R. M. Pope: Macrophages require constitutive NF-κB activation to maintain A1 expression and mitochondrial homeostasis. *Mol Cell Biol* 20, 8855-8865 (2000)

141. Rosato R. R, J. A. Almenara, S. S. Kolla, S. C. Maggio, S. Coe, M. S. Gimenez, P. Dent, S. Grant: Mechanism and functional role of XIAP and Mcl-1 down-regulation in flavopiridol/vorinostat antileukemic interactions. *Mol Cancer Ther* 6, 692-702 (2007)

142. Liu H, Q. Huang, B. Shi, P. Eksarko, V. Temkin, R. M. Pope: Regulation of Mcl-1 expression in rheumatoid arthritis synovial macrophages. *Arthritis Rheum* 54, 3174-3181 (2006)

143. Liu H, Y. Ma, S. M. Cole, C. Zander, K. H. Chen, J. Karras, R. M. Pope: Serine phosphorylation of STAT3 is essential for Mcl-1 expression and macrophage survival. *Blood* 102, 344-352 (2003)

144. Hasan Z, M. Ashraf, A. Tayyebi, R. Hussain: M. leprae inhibits apoptosis in THP-1 cells by downregulation of Bad and Bak and upregulation of Mcl-1 gene expression. *BMC Microbiol* 6, 78 (2006)

145. She Q. B, D. B. Solit, Q. Ye, K. E. O'Reilly, J. Lobo, N. Rosen: The BAD protein integrates survival signaling by EGFR/MAPK and PI3K/Akt kinase pathways in PTEN-deficient tumor cells. *Cancer Cell* 8, 287-297 (2005)

146. Antignani A, R. J. Youle: How do Bax and Bak lead to permeabilization of the outer mitochondrial membrane? *Curr Opin Cell Biol* 18, 685-689 (2006)

147. Baines C. P, R. A. Kaiser, T. Sheiko, W. J. Craigen, J. D. Molkentin: Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. *Nat Cell Biol* 9, 550-555 (2007)

148. Paquet C, E. Schmitt, M. Beauchemin, R. Bertrand: Activation of multidomain and BH3-only pro-apoptotic Bcl-2 family members in p53-defective cells. *Apoptosis* 9, 815-831 (2004)

149. Li H, H. Zhu, C. J. Xu, J. Yuan: Cleavage of BID by caspase-8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* 94, 491-501 (1998)

150. Reyland M. E: Protein Kinase C and apoptosis. In: Apoptosis, Cell signaling and Human Diseases. Ed: Srivastava R, Totowa, NJ. 2, 31-55 (2007)

151. Mellor H, P. J. Parker: The extended protein kinase C superfamily. *Biochem J* 332 (Pt 2), 281-292 (1998)

152. Monick M. M, A. B. Carter, G. Gudmundsson, L. J. Geist, G. W. Hunninghake: Changes in PKC isoforms in human alveolar macrophages compared with blood monocytes. *Am J Physiol* 275, L389-397 (1998)

153. Lin Y. F, H. M. Lee, S. J. Leu, Y. H. Tsai: The essentiality of PKCα and PKCβ translocation for $CD14^+$ monocyte differentiation towards macrophages and dendritic cells, respectively. *J Cell Biochem* 102, 429-441 (2007)

154. Aksoy E, M. Goldman, F. Willems: Protein kinase Cε: a new target to control inflammation and immune-mediated disorders. *Int J Biochem Cell Biol* 36, 183-188 (2004)

155. Brodie C, P. M. Blumberg: Regulation of cell apoptosis by Protein Kinase Cδ. *Apoptosis* 8, 19-27 (2003)

156. Garin G, J. Abe, A. Mohan, W. Lu, C. Yan, A. C. Newby, A. Rhaman, B. C. Berk: Flow antagonizes TNF-α signaling in endothelial cells by inhibiting caspase-dependent PKCζ processing. *Circ Res* 101, 97-105 (2007)

157. Basu A, D. Lu, B. Sun, A. N. Moor, G. R. Akkaraju, J. Huang: Proteolytic activation of protein kinase Cε by caspase-mediated processing and transduction of antiapoptotic signals. *J Biol Chem* 277, 41850-41856 (2002)

158. Gomel R, C. Xiang, S. Finniss, H. K. Lee, W. Lu, H. Okhrimenko, C. Brodie: The localization of Protein Kinase Cδ in different subcellular sites affects its proapoptotic and antiapoptotic functions and the activation of distinct downstream signaling pathways. *Mol Cancer Res* 5, 627-639 (2007)

159. Blass M, I. Kronfeld, G. Kazimirsky, P. M. Blumberg, C. Brodie: Tyrosine phosphorylation of protein kinase Cδ is essential for its apoptotic effect in response to etoposide. *Mol Cell Biol* 22, 182-195 (2002)

160. Voss O. H, S. Kim, M. D. Wewers, A. I. Doseff: Regulation of monocyte apoptosis by the protein kinase Cδ-dependent phosphorylation of caspase-3. *J Biol Chem* 280, 17371-17379 (2005)

161. Ross R: Atherosclerosis an inflammatory disease. *N Engl J Med* 340, 115-126 (1999)

162. Crook N. E, R. J. Clem, L. K. Miller: An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif. *J Virol* 67, 2168-2174 (1993)

163. Joazeiro C. A, A. M. Weissman: RING finger proteins: mediators of ubiquitin ligase activity. *Cell* 102, 549-552 (2000)

164. Bratton S. B, G. Walker, S. M. Srinivasula, X. M. Sun, M. Butterworth, E. S. Alnemri, G. M. Cohen: Recruitment, activation and retention of caspases-9 and -3 by Apaf-1 apoptosome and associated XIAP complexes. *Embo J* 20, 998-1009 (2001)

165. Riedl S. J, M. Renatus, R. Schwarzenbacher, Q. Zhou, C. Sun, S. W. Fesik, R. C. Liddington, G. S. Salvesen: Structural basis for the inhibition of caspase-3 by XIAP. *Cell* 104, 791-800 (2001)

166. Deveraux Q. L, R. Takahashi, G. S. Salvesen, J. C. Reed: X-linked IAP is a direct inhibitor of cell-death proteases. *Nature* 388, 300-304 (1997)

167. Deveraux Q. L, E. Leo, H. R. Stennicke, K. Welsh, G. S. Salvesen, J. C. Reed: Cleavage of human inhibitor of apoptosis protein XIAP results in fragments with distinct specificities for caspases. *Embo J* 18, 5242-5251 (1999)

168. Partheniou F, S. M. Kelsey, S. M. Srinivasula, A. C. Newland, E. S. Alnemri, L. Jia: c-IAP1 blocks TNFα-mediated cytotoxicity upstream of caspase-dependent and -independent mitochondrial events in human leukemic cells. *Biochem Biophys Res Commun* 287, 181-189 (2001)

169. Cui X, T. Imaizumi, H. Yoshida, K. Tanji, T. Matsumiya, K. Satoh: Lipopolysaccharide induces the expression of cellular inhibitor of apoptosis protein-2 in human macrophages. *Biochim Biophys Acta* 1524, 178-182 (2000)

170. Mera S, M. Magnusson, A. Tarkowski, M. Bokarewa: Extracellular survivin up-regulates adhesion molecules on the surface of leukocytes changing their reactivity pattern. *J Leukoc Biol* 83, 149-155 (2008)

171. Tamm I, S. Richter, F. Scholz, K. Schmelz, D. Oltersdorf, L. Karawajew, C. Schoch, T. Haferlach, W. D. Ludwig, C. Wuchter: XIAP expression correlates with monocytic differentiation in adult de novo AML: impact on prognosis. *Hematol J* 5, 489-495 (2004)

172. Lanneau D, M. Brunet, E. Frisan, E. Solary, M. Fontenay, C. Garrido: Heat shock proteins: essential proteins for apoptosis regulation. *J Cell Mol Med* (2008)

173. Kuppner M. C, R. Gastpar, S. Gelwer, E. Nossner, O. Ochmann, A. Scharner, R. D. Issels: The role of heat shock protein (hsp70) in dendritic cell maturation: hsp70 induces the maturation of immature dendritic cells but reduces DC differentiation from monocyte precursors. *Eur J Immunol* 31, 1602-1609 (2001)

174. Paul C, F. Manero, S. Gonin, C. Kretz-Remy, S. Virot, A. P. Arrigo: Hsp27 as a negative regulator of cytochrome C release. *Mol Cell Biol* 22, 816-834 (2002)

175. Havasi A, Z. Li, Z. Wang, J. L. Martin, V. Botla, K. Ruchalski, J. H. Schwartz, S. C. Borkan: Hsp27 inhibits Bax activation and apoptosis via a PI3 kinase-dependent mechanism. *J Biol Chem* (2008)

176. Garrido C, J. M. Bruey, A. Fromentin, A. Hammann, A. P. Arrigo, E. Solary: HSP27 inhibits cytochrome c-dependent activation of procaspase-9. *Faseb J* 13, 2061-2070 (1999)

177. Moreno-Altamirano M. M, I. Aguilar-Carmona, F. J. Sanchez-Garcia: Expression of GM1, a marker of lipid rafts, defines two subsets of human monocytes with differential endocytic capacity and lipopolysaccharide responsiveness. *Immunology* 120, 536-543 (2007)

178. Kawai T, S. Akira: Pathogen recognition with Toll-like receptors. *Curr Opin Immunol* 17, 338-344 (2005)

179. Gay N. J, M. Gangloff: Structure and function of Toll receptors and their ligands. *Annu Rev Biochem* 76, 141-165 (2007)

180. Means T. K, D. T. Golenbock, M. J. Fenton: The biology of Toll-like receptors. *Cytokine Growth Factor Rev* 11, 219-232 (2000)

181. Alexopoulou L, A. C. Holt, R. Medzhitov, R. A. Flavell: Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. *Nature* 413, 732-738 (2001)

182. Remer K. A, M. Brcic, K. S. Sauter, T. W. Jungi: Human monocytoid cells as a model to study Toll-like receptor-mediated activation. *J Immunol Methods* 313, 1-10 (2006)

183. Bosshart H, M. Heinzelmann: Lipopolysaccharide-mediated cell activation without rapid mobilization of cytosolic free calcium. *Mol Immunol* 41, 1023-1028 (2004)

184. Leber J. H, G. T. Crimmins, S. Raghavan, N. P. Meyer-Morse, J. S. Cox, D. A. Portnoy: Distinct TLR- and NLR-mediated transcriptional responses to an intracellular pathogen. *PLoS Pathog* 4, e6 (2008)

185. Doz E, S. Rose, J. Nigou, M. Gilleron, G. Puzo, F. Erard, B. Ryffel, V. F. Quesniaux: Acylation determines the toll-like receptor (TLR)-dependent positive versus $TLR2^{-}$, mannose $receptor^{-}$, and $SIGNR1^{-}$ independent negative regulation of pro-inflammatory cytokines by mycobacterial lipomannan. *J Biol Chem* 282, 26014-26025 (2007)

186. Nakagawa J, M. Saio, N. Tamakawa, T. Suwa, A. B. Frey, K. Nonaka, N. Umemura, H. Imai, G. F. Ouyang, N. Ohe, H. Yano, S. Yoshimura, T. Iwama, T. Takami: TNF expressed by tumor-associated macrophages, but not microglia, can eliminate glioma. *Int J Oncol* 30, 803-811 (2007)

187. Andreakos E. T, B. M. Foxwell, F. M. Brennan, R. N. Maini, M. Feldmann: Cytokines and anti-cytokine biologicals in autoimmunity: present and future. *Cytokine Growth Factor Rev* 13, 299-313 (2002)

188. Williams T. J, M. J. Peck: Role of prostaglandin-mediated vasodilatation in inflammation. *Nature* 270, 530-532 (1977)

189. Levy B. D, C. B. Clish, B. Schmidt, K. Gronert, C. N. Serhan: Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol* 2, 612-619 (2001)

190. Savill J, V. Fadok: Corpse clearance defines the meaning of cell death. *Nature* 407, 784-788 (2000)

191. Bhavsar M. D, M. M. Amiji: Oral IL-10 gene delivery in a microsphere-based formulation for local transfection and therapeutic efficacy in inflammatory bowel disease. *Gene Ther* 1-10 (2008)

192. Takeuchi T: Treatment of Inflammatory Immunologic Disease 3. Anti-TNF Therapy in Rheumatoid Arthritis. *Internal Medicine* 46, 1311-13112 (2007)

193. Isaacs K. L, J. D. Lewis, W. J. Sandborn, B. E. Sands, S. R. Targan: State of the art: IBD therapy and clinical trials in IBD. *Inflamm Bowel Dis* 11 Suppl 1, S3-12 (2005)

194. Wijbrandts C. A, M. G. Dijkgraaf, M. C. Kraan, M. Vinkenoog, T. J. Smeets, H. Dinant, K. Vos, W. F. Lems, G. J. Wolbink, D. E. Sijpkens, B. A. Dijkmans, P. Tak: The clinical response to infliximab in rheumatoid arthritis is in part dependent on pre-treatment TNFα expression in the synovium. *Ann Rheum Dis* (2007)

Abbreviations: Cluster of differentiation (CD), programmed cell death (PCD), Human Leukocyte Differentiation Antigens (HLDA), Major Histocompatibility Complex (MHC), Fcγ receptor (FcγR), interleukine-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), IL-8, interferon gamma (IFN-γ), Interleukine 4 (IL-4), Toll-like receptor (TLR), lipopolysaccharide binding protein (LPS), intracellular adhesion molecule 1 (ICAM-1 or CD11b), vascular cell adhesion molecule-l (VCAM-1 or CD49), granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (GM-CSF), colony stimulating factor 1 (CSF-1), caspase-recruitment domain (CARD), second zinc-binding motif RING, Baculovirus IAP repeat (BIR), Protein Kinase C δ (PKCδ), Protein Kinase B (PKB/AKT), phosphatidylserine (PS), phosphatidylserine receptor (PSR), rheumatoid arthritis (RA), Flow Cytometry Analysis (FAC), Scavenger Receptor (ScR), Mannose Receptor (MR).

Key Words: Apoptosis, Caspases, Monocytes, Macrophages, Hsp27, inflammation, survival, PKCΔ, $CD14^+$, $CD16^+$, REVIEW

Send correspondence to: Andrea I. Doseff, Heart and Lung Research Institute. The Ohio State University. 473 West 12th Ave. Columbus OH 43220 USA, Tel: 614 292-9507, Fax: 614 293-4799, E-mail: doseff.1@osu.edu

http://www.bioscience.org/current/vol14.htm