The biological role of interferon-inducible P204 protein in the development of the mononuclear phagocyte system

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TABLE OF CONTENTS

1. Abstract
2. Background
3. The mononuclear phagocyte system (MPS)
   3.1. Cellular organization
   3.2. M-CSF, a key regulator of the MPS
   3.3. M-CSF signaling and macrophage differentiation
4. Expression and control of Ifi204 in the MPS
   4.1. Pattern of expression
   4.2. Induction of expression by interferons and cytokines
5. Role of Ifi204 in MPS development
   5.1. Negative growth control
   5.2. Positive control of differentiation
6. Summary and perspectives
7. Acknowledgments
8. References

1. ABSTRACT

The mononuclear phagocyte system (MPS) is a cell population derived from progenitor cells in the bone marrow, and comprising monocytes, macrophages, osteoclasts, dendritic cells, and microglia. Homeostasis of the MPS and response to physiological stress is under the control of signaling molecules and nuclear factors; among them, macrophage-colony-stimulating factor (M-CSF) controls monocyte/macrophage lineage development. Here we discuss the implication of Ifi204, a M-CSF-responsive gene, in the proliferation and differentiation of monocytes/macrophages. Ifi204 is a member of the interferon-inducible p200 family of proteins, and was found to be an important regulator of differentiation of both skeletal and cardiac muscles and osteogenesis. Ifi204 is expressed at the early stages of differentiation of MPS cells and later in the monocyte/macrophage lineage. IFI16, the closest Ifi protein in human, is expressed all along the monocytic lineage. In MPS cells, Ifi204 expression is induced by interferons but also by various stimuli, independently of the presence of interferon. Enforced expression of p204 in interleukin-3 (IL3)-dependent FD-Fms cell line strongly decreased both IL3- and M-CSF-dependent proliferation and conversely favored macrophage differentiation of FD-Fms cells in response to M-CSF. Altogether, data enlighten a role of Ifi204 as a regulator of monocyte/macrophage differentiation and make possible a connection with other myeloid regulators.

2. BACKGROUND

Interferons (IFNs) play an essential role in host defence against viral and bacterial pathogens. They induce the expression of many cellular genes, the IFN responsive genes, whose products can interfere both with virus replication and growth and survival of host cells. HIN-200 protein family represents a group of proteins featuring Hematopoetic expression, IFN inducibility, Nuclear localization, and strongly conserved 200 amino-acid repeats at their C-termini. These proteins are associated with cell cycle arrest and sensitize cells to apoptosis, which can contribute to anti-viral responses. The members of this protein family show different and specific patterns of expression, as well as inducibility by other cytokines than IFN, suggesting that each member might be endowed with important regulatory functions in tissue development and differentiation. Here we review recent data from the litterature indicating a role of p204, the product of the murine Ifi204 gene, and its human homolog IFI16, in the production of monocytes and cellular components of the mononuclear phagocyte system.

3. THE MONONUCLEAR PHAGOCYTE SYSTEM (MPS)

3.1. Cellular organization

Macrophages constitute an important cell population in most tissues of the body. They play major
roles in homeostasis, infection, tissue repair, and tumor development. Macrophages are known to mainly arise from the maturation of monocytes that are generated from myeloid progenitor cells in the bone marrow and have the ability to rapidly enter tissues once they have been released in the blood (1, 2). This cellular system was originally termed mononuclear phagocyte system (MPS), but recent data have highlighted its marked heterogeneity and plasticity (3, 4). It is now admitted that mononuclear phagocytes encompass related cells exhibiting highly specialized functions, such as macrophages, osteoclasts, myeloid dendritic cells, and microglia (Figure 1). The common view holds that mononuclear phagocytes are derived from circulating monocytes. Thus, monocytic cells differentiate into osteoclasts in response to macrophage colony-stimulating factor (M-CSF) and receptor activator of NFκB ligand (RANKL) (5, 6). Similarly, dendritic cells can be derived from circulating monocytes following appropriate stimulation (7, 8). Although the precise origin of microglia is still strongly debated, data support its monocytic origin at least at certain stages of development (9). The single origin of mononuclear phagocytes is challenged by the now well-established monocytic heterogeneity (3, 10). However, there are growing evidences that subpopulations of blood monocytes differ in their maturation stage and functional properties rather than they represent separate cell lineages (10, 11). In the bone marrow, common progenitors to macrophages, osteoclasts, and dendritic cells have been identified (12-14), as well as microglia progenitors of hematopoietic origin (15-17). Nonetheless, the question is still open as to whether there is a specific bone marrow progenitor for the MPS. Interestingly, ex vivo expansion of monocytic cells in Flt3+ stimulated cultures of mouse bone marrow cells results in the sequential appearance of precursors of osteoclasts, dendritic cells, and microglia, which may account for successive waves of lineage commitment (18).

Collectively, studies provide a consensus view that cells of the MPS are derived from monocyte progenitors whose terminal differentiation to either macrophages, osteoclasts, dendritic cells, or microglia would be dictated by the numerous extrinsic factors they are subjected to during their development from myeloid progenitors and migration through blood vessel wall and into tissues (3, 19).

3.2. M-CSF, a key regulator of the MPS
M-CSF (or CSF-1, for colony-stimulating factor-1) was originally defined as the growth factor that enables in vitro development of macrophage colonies from bone marrow progenitors (20). Further studies, both in vitro and in vivo, have shown that M-CSF is strongly implicated all along the MPS. Frameshift mutation prevents production of active protein from the M-CSF gene in osteopetrosis mice that exhibit severe deficiencies in macrophages, osteoclasts, microglia, and dendritic cell subsets, including Langerhans cells (21-25). In vitro, M-CSF is a survival, activation and proliferation factor for mature macrophages and microglia (26, 27). It is also a survival factor for osteoclasts (28) and

![Figure 1. Schematic representation of the hematopoietic system with emphasis on the MPS. HSC, hematopoietic stem cell; CMP, common myeloid progenitor; CLP, common lymphoid progenitor; GMP, granulocyte and macrophage progenitor; MEP, megakaryocyte and erythrocyte progenitor.](image-url)
Ifi204 in macrophage differentiation

facilitates their development through stimulation of RANK expression (12). Finally, M-CSF was recently shown to modulate dendritic cell differentiation (25, 29) and to promote their transdifferentiation to osteoclasts (30).

The macrophage-colony stimulating activity of M-CSF suggested that macrophages, and possibly other cells from the MPS, originate from a lineage-specific progenitor cell, called colony-forming unit-macrophage (CFU-M). Rather, it seems more likely that bipotential myeloid progenitors are the primary target for M-CSF in the bone marrow. Indeed, commitment of purified granulocyte-macrophage colony-forming cells (GM-CFC) that normally give rise to colonies comprising both neutrophils and macrophages is totally shifted to the macrophage lineage by M-CSF, suggesting that the unique properties of M-CSF to stimulate macrophage colony development result from instructive signaling on bipotential myeloid progenitors (31).

3.3. M-CSF signaling and macrophage differentiation

Consistent with the biological functions of M-CSF, M-CSF receptor (M-CSFR), the product of the c-fms proto-oncogene, is expressed at all stages of the MPS in vivo (32). M-CSFR is a tyrosine kinase receptor closely related to c-Kit, Flt3 and PDGF receptors. M-CSF binding to M-CSFR induces dimerization and conformational changes in the cytoplasmic region of the receptor, resulting in phosphorylation of specific tyrosine residues. These autophosphorylation sites serve as anchoring points for signaling molecules with SH2 domains, including Src family kinases, Mono/Gads and Grb2 adapters, phosphatidylinositol 3’ kinase, and phospholipase Cgamma2 (27, 33, 34). Moreover, the scaffolding protein Gab2 is rapidly phosphorylated in response to M-CSF and is thereby able to recruit and activate PI 3-kinase and tyrosine phosphatase SHP2 (35). Therefore, activation of M-CSFR results in the assembly of specific multimere complexes that initiate multiple downstream signaling pathways that are thought to cooperate to regulate gene expression.

The multiple roles assigned to M-CSF in development and functions of the MPS pose the question of how the signaling pathways evoked by M-CSFR activation can lead to such different cellular responses as survival, proliferation and differentiation in various cellular contexts. Several groups, including ours, have focused their research on the mechanisms by which M-CSF regulates myeloid progenitor cell commitment to the macrophage lineage, an early developmental step in myelopoiesis likely shared by all cells of the MPS. It was found that commitment to macrophage requires persistent and elevated activation of the MAPK ERK and ERK2 (36-39). However, few studies have searched for genes specifically activated in response to M-CSF during this commitment phase. In an attempt to isolate such M-CSF-responsive genes, we used an in vitro gene trap strategy, which led us to identify a novel player in macrophage differentiation, p204, the product of the Ifi204 gene (40).

4. EXPRESSION AND CONTROL OF IFI204 IN THE MPS

4.1. Pattern of expression

Ifi204 is a member of the cluster of murine HIN-200 genes that encode interferon-inducible p200 family proteins. p204 is a 72 kDa phosphoprotein that contains two partially conserved 200 amino acid segments, designed as type a and type b, bearing a LXCXE motif that enables p204 binding to the retinoblastoma protein (pRb) and mediates its antiproliferative activity (41-43). In addition, p204 can inhibit cell growth by inhibiting rRNA transcription after binding to UBF1 transcription factor (44). Another region conserved among most of the p200 family proteins is the PAAD/DAPIN/Pyrin domain located at the N-terminus. The secondary structure of this domain consists of five alpha-helices and functions as a putative protein-protein interaction domain (45). Based on protein structure similarities, the closest Ifi protein to p204 in human is presumably IFI116 (45), yet IFI116 may function differently from p204 since it negatively regulates both the pRb and the p53 pathways, like murine p202 (46).

Expression of Ifi204 is detected during heart and skeletal muscle differentiation and osteogenesis (47-49). In the hematopoietic system, p204 is detected in bone marrow, thymus and lymph node (50). During lymphopoiesis, expression of Ifi204 transcript is upregulated by Notch1 signaling when thymocytes mature in single-positive cells, suggesting a possible role of p204 in T lymphocyte differentiation (51). Concerning myelopoiesis, several studies have enlightened the relationships between Ifi204 expression and monocytic development.

Transcriptional profiling during commitment to granulocyte and monocyte differentiation of progenitors controlled by conditional version of the E2a/Pbx1 oncogene has revealed gene expression signature distinguishing granulocyte from monocyte pathways; thus, Ifi204 was expressed exclusively in monocyte progenitors and monocytic cells (52). In vivo, p204 protein is specifically expressed in Mac-1+ cells from the peripheral blood leukocytes and bone marrow cells. Resident peritoneal macrophages do not express p204 but expression of the protein is strongly induced after treatment of mice with an IFN-inducer, the synthetic dsRNA poly rI :rC treatment (50). At variance, significant expression of Ifi204 could be detected in macrophages obtained in cultures of peritoneal (50) or bone marrow-derived macrophages (40) in the absence of IFN stimulation.

In the human hematopoietic system, IFI116 is expressed in mononuclear leukocytes (monocytes and lymphocytes), but not in granulocytes, in contrast to another HIN200 family member, MND2, that is expressed in both granulocytes and monocytes. Moreover, uncommitted CD34+ hematopoietic progenitor cells constitutively express IFI116, and this expression is maintained during their differentiation along monocytic lineage, whereas it is strongly down-regulated after commitment toward the granulocytic pathway (53, 54).
Ifi204 in macrophage differentiation

Figure 2. Ifi204 induction during M-CSF-induced macrophage differentiation of pro-T EGER-Fms cells. Ifi204 and MafB transcript expressions were analysed by RT-PCR using total RNA isolated from EGER-Fms cells maintained in the presence of IL7, SCF, and estradiol (day 0), or shifted to M-CSF-containing medium in the absence of estradiol for 1, 2, 3, 5, 7 or 9 days; HPRT transcript expression was used as a loading control.

We have shown that Ifi204 is not expressed in several murine myeloid progenitor cell lines, including 32Dcl23, FDC-P1, M1 cells, whereas it is significantly expressed in four different monocyte/macrophage cell lines (40). Consistent with this observation, Ifi204 expression is induced when progenitor cell lines are stimulated to differentiate to macrophages (40). Using a more immature cell line (55), we have recently observed that Ifi204 induction is an early event that precedes the upregulation of key regulators of macrophage differentiation such as the bZip transcription factor MafB (56) (Figure 2). Although it remains to determine if Ifi204 is expressed in native immature hematopoietic cells, it appears that, unlike IFI16, Ifi204 expression seems to be restricted to committed progenitors, reinforcing the idea that Ifi204 and IFI16 may have different functions in MPS cells.

4.2. Induction of expression by interferons and cytokines

Multiple studies have demonstrated that Ifi204 transcripts are inducible by IFNs in various cell types, including fibroblasts, epithelial, or hematopoietic cells (50, 57). It now appears that Ifi204 expression can also be induced by various stimuli in an IFN-independent manner. Thus, LPS or fetal bovine serum growth factors are able to activate Ifi204 expression in cultured macrophages (50). Moreover, LPS, but not IFN, is able to induce Ifi204 (and also Ifi202 and Ifi205) expression in macrophages derived from C57BL/6 mice bone marrow cells, suggesting activation of distinct transcriptional mechanisms by IFN and LPS.

Monocytic progenitors and their progeny specifically express IRF5, IRF7, and IRF8 as well as a set of 8 additional interferon-inducible genes among which Ifi204, suggesting that this set of genes is transcriptionally co-regulated, potentially by IRF transcription factors (52). IRF8 (or ICSBP interferon consensus sequence binding protein) is a good candidate since it is active in myeloid progenitor cells and positively regulates macrophage differentiation (58, 59). Persistent activation of an interferon signaling pathway may be ruled out because other IFN responsive genes are not expressed, such as those encoding macrophage IFN-inducible protein 10, IFNgamma-inducible monokine, or IFN-induced kinase (52).

In the interleukin-3 (IL3)-dependent FD-Fms cells that differentiate in macrophages in response to M-CSF, induction of Ifi204, together with that of Ifi203, is IFN-independent since it was observed in the presence of a cocktail of IFN-neutralizing antibodies (40). However, Ifi204 induction myeloid progenitor cell lines is not limited to M-CSF since it was observed in M1 myeloid cells stimulated with leukemia inhibitory factor (LIF). In FD-Fms cells, M-CSF and IFN-γ activate distinct signaling pathways to induce Ifi204 expression, the MAPK and Src signaling pathways, and the PI3K signaling pathway, respectively.

Finally, similar IFN-independent induction of IFI16 has been reported in human myeloid differentiation. Study of the transcriptional program activated by the p210 BCR/ABL1 fusion oncogene revealed a set of IFN-responsive genes, including IFI16, that are expressed in the absence of autocrine IFN production. Thus, BCR/ABL1 tyrosine kinase may partly signal through similar pathways as IFN, leading to the expression of a similar subset of genes in monocytic cells (60) or, alternatively use a specific pathway as observed with M-CSF in FD-Fms cells (40).

5. ROLE OF IFI204 IN MPS DEVELOPMENT

5.1. Negative growth control

The role of Ifi204 as a growth regulator has been investigated by constitutive expression of p204 into various cell lines, including fibroblasts (41, 61), osteosarcoma (62), or IL3-dependent myeloid cells (63). In most cell lines, Ifi204 strongly impaired cell growth, leading to the view that proliferation arrest may explain the failure to obtain transgenic mice constitutively expressing p204 (61). However, it is of note that two cell lines expressing high level of ectopic p204 have been described as being refractory to p204-induced growth inhibition (61).

Since Ifi204 was induced by M-CSF during macrophage differentiation of FD-Fms cells when cells decreased their growth rate, we investigated the growth inhibition effect of Ifi204 in this model. Constitutive Ifi204 expression in FD-Fms cells strongly inhibits both IL3 or M-CSF-dependent proliferation (40). The mechanisms by which p204 promotes growth arrest of myeloid progenitor cells remain to be precised. Nonetheless, we may presume that negative regulation of pRb is involved, as previously shown in fibroblasts. Indeed, p204 was unable to induce cell growth arrest in Rb-/- murine embryo fibroblasts or Rb-deficient fibroblast cell lines, and its anti-proliferative effect could be abolished by deleting its Rb-binding domains (42). However, p204, like p205, is also able to inhibit cell growth in a p53- and pRb-independent manner in osteosarcoma cell lines (62).
Ifi204 in macrophage differentiation

5.2. Positive control of differentiation

p204 promotes the differentiation of skeletal muscle myotubes, cardiac myocytes, and osteoblasts (47-49, 64-66). Enforced Ifi204 expression had a tremendous effect on macrophage differentiation of FD-Fms cells in response to M-CSF, resulting in fully differentiated macrophage-like morphology of all cells expressing the p204 protein (40). While searching for myeloid markers that are induced by p204 overexpression, we found that the myeloid-specific transcription factor Egr-1 was induced in IL3-stimulated FD-Fms cells, independently of the presence of M-CSF. Egr-1 is a zinc finger transcription factor that is implicated in macrophage differentiation (67, 68) and is induced by M-CSF in FD-Fms cells (40). Although the precise role of Egr proteins in myeloid differentiation remains to be determined (69), Egr-1 expression is clearly a part of the macrophage differentiation program (70). The ability of p204 to induce this primary myeloid differentiation gene in the absence of M-CSF strengthens the idea that p204 may drive molecular components of the macrophage differentiation program.

6. SUMMARY AND PERSPECTIVES

Expression of most of HIN-200 family proteins appears to be stage- and lineage-specific, indicating that it is tightly regulated during hematopoietic development and points to possible functional roles during differentiation process. Recent studies strongly support the role of p204 as a regulator of cell proliferation and differentiation in the MPS. However, the primary molecular targets of p204 during macrophage development remain to be determined. Other proteins than pRB may contribute to negative regulation of cell growth by p204 and in the absence of M-CSF, p204 protein (40). While searching for myeloid markers that are induced by p204 overexpression, we found that the myeloid-specific transcription factor Egr-1 was induced in IL3-stimulated FD-Fms cells, independently of the presence of M-CSF. Egr-1 is a zinc finger transcription factor that is implicated in macrophage differentiation (67, 68) and is induced by M-CSF in FD-Fms cells (40). Although the precise role of Egr proteins in myeloid differentiation remains to be determined (69), Egr-1 expression is clearly a part of the macrophage differentiation program (70). The ability of p204 to induce this primary myeloid differentiation gene in the absence of M-CSF strengthens the idea that p204 may drive molecular components of the macrophage differentiation program.

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Ifl204 in macrophage differentiation


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