

Development and Differentiation of Macrophages and Related Cells : Historical Review and Current Concepts

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Two major theories concerning the development and differentiation of macrophages — the reticuloendothelial system proposed by Aschoff (1924) and the mononuclear phagocyte system developed by van Furth (1972) — are critically reviewed. Phylogenetically, mononuclear phagocytic cells (macrophages) develop in all animals; monocytes are not detected in invertebrates, and both macrophages and monocytes appear in vertebrates. The phylogenetic principle that the development and differentiation of macrophages precede those of monocytes during the evolutionary processes of animals applies to human and murine ontogeny of macrophages. In early ontogeny, macrophages develop from hematopoietic stem cells during yolk sac hematopoiesis, and the stage of monocytic cells is bypassed. Monocytic cells develop during hepatic hematopoiesis, and their development proceeds from the middle stage of ontogeny.

In postnatal and adult life, macrophages are differentiated from macrophage precursor cells at different stages or through different pathways of differentiation. In addition to developing via the differentiation pathway of monocytic cells into macrophages, tissue macrophages develop from macrophage precursor cells at or before the stage of granulocyte/macrophage colony-forming cells, and some macrophage populations are derived from B lymphoid precursor cells. Dendritic cells are also derived from different precursor cells and are classified into myeloid dendritic cells, monocyte-derived dendritic cells, and lymphoid dendritic cells according to their precursor cell origin. Thus, macrophages and their related cells are believed to be differentiated from hematopoietic stem cells through multiple pathways. Finally, the roles of two major macrophage populations, Kupffer cells and monocyte-derived macrophages, in hepatic granuloma formation are analyzed by considering various mouse models.

Key words macrophages, reticuloendothelial system, mononuclear phagocyte system, monocytes, dendritic cells

INTRODUCTION

Macrophages are a heterogeneous population of cells ubiquitously distributed in various organs and tissues of humans and animals; they show different cell morphology and have variable functions according to requirements of the tissues in which they occur. More than a century has already passed since Metchnikoff in 1892 first termed large phagocytic cells “macrophages” on the basis of his phylogenetic studies and described their presence in all invertebrates and

vertebrates¹. During that time, the origin and differentiation of macrophages and their related cells have been seriously debated. In 1924, Aschoff proposed the concept of the reticuloendothelial system (RES) and included macrophages (histiocytes) as a major member of this system, together with reticulum cells and reticuloendothelia (phagocytic endothelia)^{2,3}. In contrast to this theory, van Furth and colleagues presented the concept of the mononuclear phagocyte system (MPS) and maintained that all macrophages, not only those appearing in inflammatory foci but also those residing in tissues under normal steady-state conditions, are derived from monocytes, which differentiate via promonocytes

Table 1. Reticuloendothelial system (RES : Aschoff, 1924)

RES in the broad sense	RES in the narrow sense	1) Vascular endothelial cells (blood and lymphatic vessels)
		2) Fibrocytes
		3) Reticulum cells : Spleen (pulp), lymph nodes (lymph follicles and medulla), lymphatic tissues
		4) Reticuloendothelia : Lymph nodes (lymph sinus), spleen (venous sinus), hepatic sinusoids (Kupffer cells), bone marrow, adrenal cortex, and pituitary gland (capillaries)
		5) Histiocytes : Connective tissues
		6) Splenocytes, pigment-ingesting monocytes

from monoblasts originating in bone marrow^{4,5}. According to this concept, blood monocytes have no proliferative capacity and macrophages are considered to be short-lived, nondividing terminal cells of the MPS. However, data conflicting with the concept of MPS have been presented. According to both phylogenetic and ontogenetic viewpoints, macrophages emerge before the development of monocytic cells, which contradicts the MPS view that all macrophages are derived from monocytes. Also in conflict with the concept of MPS, studies have shown the differentiation pathways of macrophages from hematopoietic stem cells without passage through the developmental stages of monocytic cells. Some macrophage populations develop from hematopoietic stem cells via common lymphoid progenitors.

In this article, the author reviews the concepts of RES and MPS and their related experimental data and discusses the current concept of the development and differentiation of macrophages and their related cells through multiple pathways of differentiation from their progenitor cells.

I. Previous theories of development and differentiation of macrophages and related Cells

1. Reticuloendothelial system and criticism of this concept

In 1924, Ludwig Aschoff determined that reticulum cells, reticuloendothelia (phagocytic endothelia), and histiocytes (tissue macrophages) show positive reactions for vital staining with lithium carmine; he regarded them as a single cell system of local tissue origin and proposed the concept of the RES^{2,3} (Table 1). Also, he recog-

Table 2. Histiocytic cell system (Kiyono, 1918)

Histiocytes
Reticuloendothelial cells
Reticulum cells
Reticuloendothelia (phagocytic endothelia)

nized these three types of cells as phagocytic cells and considered that reticulum cells become flattened and show a shape like that of endothelial cells, when they are located at sites facing the blood or lymph stream. These phagocytic endothelial cells were called "reticuloendothelia". Furthermore, he thought that reticulum cells and reticuloendothelia transform into histiocytes as free, round phagocytic mononuclear cells. All these cells were generally called "reticuloendothelial cells" (RE cells). The RES theory was based on the results of his studies with his co-workers, particularly vital staining studies with Kiyono in 1914-1918^{6,7} and cholesterol metabolism studies with Landau and McNee in 1914⁸. Before Aschoff's postulate of the RES concept, Kiyono proposed the histiocytic cell system, which included histiocytes, reticulum cells, and reticuloendothelia (Table 2); this view provided a basis for the RES theory of Aschoff. Therefore, the RES theory was also called "the theory of Aschoff and Kiyono". This fascinating theory was widely accepted for nearly half a century, until the end of the 1960s.

However, Aschoff's concept of the RES had four major paralogs. First, he considered that all RE cells were derived from local tissue cells and that reticulum cells, histiocytes, and reticuloendothelia were related in terms of their origin. However, he had not confirmed the mutual relationship of these cells. Akazaki, a

Japanese pupil of Aschoff, extensively studied the distribution, ontogenesis, and origin of RE cells, in collaboration with many Japanese co-workers at both Niigata and Tohoku Universities, for 30 years⁹. In 1952, Akazaki concluded that RE cells were composed of two different groups of cells, 1) reticuloendothelia and 2) reticulum cells or histiocytes, and that reticuloendothelia had an endothelial cell origin, which was thus different from that of reticulum cells or histiocytes^{3,9}. Afterward, Kojima, a pupil of Akazaki, also actively studied the relationship between reticulum cells and histiocytes (tissue macrophages), in collaboration with many co-workers mainly at Fukushima Medical College, for 20 years and concluded that histiocytes differed from reticulum cells in cell morphology, function, and origin¹⁰.

Second, with regard to RE cells being positive for vital staining with lithium carmine, Aschoff believed that this compound was taken up by the RE cells through phagocytosis ("cell eating"). In 1932, Lewis first presented evidence that all living cells can ingest various materials by pinocytosis ("cell drinking")¹¹. In distinction to phagocytosis by macrophages, reticulum cells and endothelial cells can also take up lithium carmine by pinocytosis. Third, repeated intravenous injections of lithium carmine stimulate animals. Fourth, this chemical compound accumulates within lysosomes because it is not degraded therein; the concept of lysosomes was first established by de Duve and co-workers in 1956¹², about 30 years after proposal of the RES concept by Aschoff. As for vital staining, current understanding is that lithium carmine is taken up by reticulum cells and endothelial cells through pinocytosis, is not degraded in lysosomes, and is accumulated in massive amounts in large lysosomal granules. The large lysosomal granules of these RE cells were mistaken for evidence of the phagocytosis usually observed in macrophages.

From the 1950s on, various prominent advances in technology occurred in the field of histochemistry, and the methodologies of immunohistochemistry, electron microscopy, and immunology developed. Numerous studies using these advanced technologies provided evidence that macrophages are distinct from reticulum cells and endothelial cells, so that by the end of the 1960s, it was recognized that macrophages,

reticulum cells, and endothelial cells differ from each other in morphology, function, and origin.

2. Monocyte origin of macrophages

In 1925, Sabin *et al.* in their studies of supravital staining reported the presence of two types of macrophages in connective tissues, and they emphasized that macrophages were derived from monocytes originating from progenitor cells in bone marrow¹³. In 1939, during studies of a chamber made in the ear of rabbits, Ebert and Florey found that macrophages in inflammatory foci were derived from monocytes migrating from peripheral blood *in vivo*¹⁴. In 1948, Amano, a Japanese hematopathologist at Kyoto University, also maintained on the basis of his studies with supravital staining that macrophages in inflammatory foci or normal steady-state conditions were derived from monocytes migrating from peripheral blood and that monocytes developed via promonocytes from monoblasts originating from progenitor cells in bone marrow¹⁵. In the 1960s, the development and differentiation of macrophages were studied by various methods, including radiation-induced chimeras, skin windows, parabiosis, chromosome markers, cytochemistry, and autoradiography with [³H] thymidine. Studies showed that macrophages not only in inflammatory foci but also in normal steady-state conditions are differentiated from blood monocytes originating in bone marrow.

3. Mononuclear phagocyte system and its experimental basis

In 1969, Langevoort, Cohn, Hirsh, Humphrey, Spector, van Furth, and many other American and European researchers had an international meeting at Leiden, rejected the concept of the RES, and selected mononuclear phagocytic cells (mononuclear phagocytes) alone as the basis for the MPS, excluding reticulum cells, fibroblasts, endothelial cells, and other nonphagocytic mesenchymal cells¹⁶. These investigators proposed the concept of the MPS: all macrophages are derived from monocytes, which differentiate through promonocytes from monoblasts originating in bone marrow^{4,5,16}. van Furth promoted the view that macrophages are nondividing, short-lived, terminal cells of the MPS and are supplied from blood monocytes alone¹⁶⁻²⁰. van Furth included,

Table 3. Mononuclear phagocyte system (MPS : van Furth, 1980)

Hemopoietic stem cells	
↓	
Committed stem cells	
↓	
Monoblasts	BONE MARROW
↓	
Promonocytes	
↓	
Monocytes	
↓	
Monocytes	PERIPHERAL BLOOD
↓	
Macrophages	TISSUES
Normal steady state	Inflammation
Connective tissue (histiocytes)	Exudate macrophages
Liver (Kupffer cells)	Exudate-resident macrophages
Lungs (alveolar macrophages)	Epithelioid cells
Lymph nodes (free and fixed macrophages, interdigitating cells?)	Multinuclear giant cells (Langhans type, foreign body type)
Spleen (free and fixed macrophages)	
Bone marrow (fixed macrophages)	
Serous cavity (pleural and peritoneal macrophages)	
Bone (osteoclasts)	
Skin (histiocytes ; Langerhans cells?)	
Synovia (A cells)	
Other tissues (tissue macrophages)	

as members of the MPS, besides monocyte-derived macrophages in inflammatory foci, tissue macrophages (Kupffer cells in the liver ; free or fixed macrophages in the spleen, lymph nodes, bone marrow, and thymus ; histiocytes in the skin and connective tissues ; free macrophages in the body cavities ; and macrophages in other various tissues), osteoclasts in bone, and microglia in the brain under normal steady-state conditions. In addition, dendritic cells, such as epidermal Langerhans cells and interdigitating cells in lymph nodes and lymphoid tissues, were included as possible members of the system (i. e., with a question mark)⁵ (Table 3).

The essential idea of the MPS was based on the results obtained from studies, using [³H] thymidine autoradiography, of 1) radiation-induced chimeras, 2) parabiosis, and 3) monocytopenia induced by administration of glucocorticoids in massive doses. 1) In radiation-induced chimeras, bone marrow cells were transplanted into mice or rats after whole-body irradiation with or without partial bone marrow shielding, and cells were tracked via chromosome markers, esterase staining, and specific antisera against macrophages to examine infiltration of bone marrow cells into tissues and their differen-

tiation *in loco*. By these methods, transplanted bone marrow cells were demonstrated to differentiate into monocytes, infiltrate tissues, and become macrophages⁴. However, these experiments provided no evidence that monocytes differentiate into tissue macrophages, because whole-body irradiation causes marked damage in all organs and tissues, the reasonable result being that monocytes infiltrate damaged tissues or inflammatory lesions and differentiate into macrophages *in loco*. 2) On the basis of results of experiments on parabiosis obtained in a personal communication from Parwaresch, Crofton, Diesselhoff-den Duck and van Furth concluded that Kupffer cells, tissue macrophages in liver, were derived from monocytes²¹. However, conflicting results were presented²²⁻²⁷, and three groups of investigators independently denied the monocytic origin of tissue macrophages²⁵⁻²⁷. 3) Daily administration of massive doses of glucocorticoids into mice induces monocytopenia, as well as a severe reduction in the number of tissue macrophages^{19,28,29}. van Furth interpreted this numerical reduction in macrophages in various tissues as resulting from monocytopenia^{19,28,29}, because tissue macrophages were believed to be a nondividing cell

population constantly supplied from blood monocytes^{4,5,16-19}. However, the proliferative capacity of tissue macrophages escaped his notice. As will be mentioned below, tissue macrophages clearly possess a proliferative capacity. Macrophage proliferation was demonstrated to be severely impaired by administration of corticosteroid hormones³⁰⁻³¹.

Although the MPS theory has greatly contributed to definition of the differentiation pathway of monocytic cells into macrophages in inflammatory lesions, whether all tissue macrophages are derived from monocytes alone in the normal steady state remains a question^{10,32-36}.

4. Criticism of the mononuclear phagocyte system theory

1) Two major subpopulations of macrophages

In 1972, Daems and Brederoo used enzyme cytochemical studies of peritoneal macrophages in guinea pigs to demonstrate the presence of two subpopulations of macrophages that differed in the localization pattern of endogenous peroxidase (PO), which allowed discrimination of exudate macrophages from resident macrophages³⁷. Like monocytes, exudate macrophages show PO activity in cytoplasmic granules alone that is resistant to aminotriazole treatment, and they appear in stimulated inflammatory foci. In contrast, resident macrophages show PO activity in the nuclear envelope and rough endoplasmic reticulum and usually exist in unstimulated, normal steady-state conditions^{37,38}. Daems and his co-workers and Kojima and his colleagues studied rats, mice, and humans, in addition to guinea pigs, to demonstrate the existence of resident macrophages in various tissues under unstimulated, normal steady-state conditions^{10,38-42}. In the following cells, they found PO activity in a location similar to its location in peritoneal resident macrophages: pleural macrophages; alveolar macrophages in lungs; Kupffer cells in the liver; histiocytes in dermal, subcutaneous, and interstitial connective tissues; macrophages in the thymus, lymph nodes, and other peripheral lymphoid tissues; medullary macrophages in bone marrow; and macrophages in many other tissues. Kojima in 1976 believed these resident macrophages to be a major subpopulation residing in various tissues and grouped them in a

family of histiocytes or tissue macrophages¹⁰.

In 1977-1978, Bodel *et al.* in their *in vitro* studies of rabbit blood monocytes found transient expression of PO activity in the nuclear envelope and rough endoplasmic reticulum of monocytes after adherence of the cells to glass slides; they emphasized that these cells are a transitional or intermediate form between exudate macrophages and resident macrophages^{43,44}. This phenomenon was also demonstrated in mice, rats, guinea pigs, and humans⁴⁵⁻⁴⁹, and this type of cell was termed an "exudate-resident" macrophage, thus adding evidence to support the concept of the MPS: monocytes differentiate into exudate macrophages, which become resident macrophages by passing through the stage of exudate-resident macrophages⁵. Beelen *et al.*, Bainton, and Watanabe *et al.* found confirmation of the differentiation pathway of monocytes into tissue macrophages in their *in vivo* studies⁴⁵⁻⁴⁹. However, other investigators did not agree with this pathway for the following reasons: 1) the exudate-resident macrophages have never been found in tissues under unstimulated normal steady-state conditions⁵⁰; 2) the expression of PO activity in the nuclear envelope and rough endoplasmic reticulum of exudate-resident macrophages is a transient phenomenon distinct from the stable and constitutive expression of PO activity usually found in resident macrophages^{50,51}; 3) biochemical properties of PO differ from exudate macrophages to resident macrophages: exudate macrophages contain myeloperoxidase in the cytoplasmic granules, whereas the PO in the nuclear envelope and rough endoplasmic reticulum has cytochemical characteristics of catalase^{50,52,53}; and 4) exudate-resident macrophages are indistinguishable from resident macrophages that have taken up PO-positive granules released from monocytes or exudate macrophages^{50,54,55}.

Using the double staining method of PO cytochemistry and ultrastructural immunocytochemistry with anti-rat macrophage monoclonal antibodies ED1, ED2, and ED3, Beelen *et al.* demonstrated that ED1-positive monocytes and exudate macrophages differentiated into ED2-positive resident macrophages via ED3-positive exudate-resident macrophages⁵⁶. In contrast, in our previous studies using the same method of PO cytochemistry and ultrastructural immunocytochemistry with two different anti-rat macro-

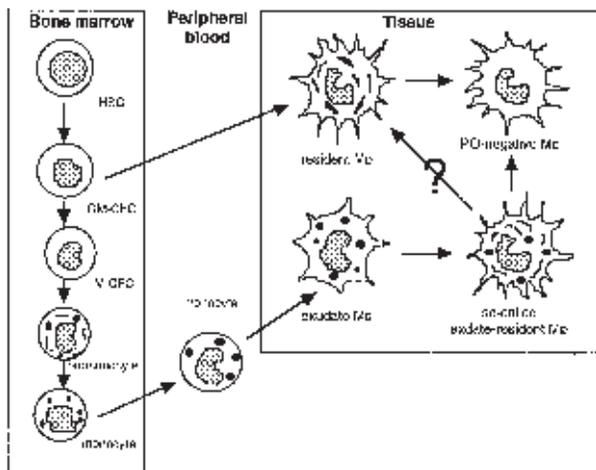


Fig. 1. Schematic diagram of the relationship between the monocyte-derived macrophages or tissue (resident) macrophages and their precursor cells (HSC: hematopoietic stem cell, GM-CFC: granulocyte/macrophage colony-forming cell, M-CFC: macrophage colony-forming cell, $M\phi$: macrophage).

phage monoclonal antibodies, Ki-M2R and TRPM-3, we clearly demonstrated that TRPM-3 (sialoadhesin, CD169) recognizes exudate macrophages and so-called exudate-resident macrophages but not resident macrophages, whereas Ki-M2R is reactive for resident macrophages and not for exudate macrophages and exudate-resident macrophages⁵⁷. Also, PO-negative macrophages consist of two different subpopulations: one is positive for TRPM-3 and the other is positive for Ki-M2R⁵⁷. Results of our studies therefore indicate that the so-called exudate-resident macrophages are not a transitional or intermediate form between exudate macrophages and resident macrophages. In addition, tissue macrophage precursors do not show PO activity in any cytoplasmic organelles and belong to a PO-negative cell subpopulation⁵⁸. Fig. 1 provides a schematic diagram of the relationship between monocyte-derived macrophages or tissue macrophages and their precursor cells.

2) Proliferative capacity of macrophages

van Furth reported that the cell cycle of monoblasts in mice was 11.9 h, a monoblast gave rise to two promonocytes via cell division, and a promonocyte divided into two monocytes within 16.2 h^{6,18-20}. Monocytes lose proliferative capacity; they are released from bone marrow within 24 h after maturation, circulate in peripheral blood, and enter peripheral tissues^{6,18-20}. Accord-

ing to the MPS concept, macrophages also have no proliferative capacity, as evidenced by the negligible [³H] thymidine uptake of macrophages (below 5%) and because a small number of proliferating cells are promonocytes that have just arrived at the tissues from the bone marrow^{6,18-20}.

In contrast, it has been repeatedly reported that macrophages have proliferative capacity. Parwaresch and Wacker reported in 1984 in their experimental study of parabiosis that half of the peritoneal macrophages can survive by cell division²¹. Volkman and his co-workers developed a severely monocytopenic mouse model by use of a single intravenous injection of strontium-89 (⁸⁹Sr)⁵⁹⁻⁶¹. In these mice, within 1 h after injection, this radioisotope is incorporated into bone tissues by an exchange with calcium, accumulates in bone tissues, and irradiates and destroys bone marrow without any damage to peripheral tissues distant from the bones. From 2 weeks after injection, monocytes completely disappear in peripheral blood, and severe monocytopenia continues for a long time, 10 weeks or more. In severely monocytopenic mice, however, numbers of tissue macrophages such as peritoneal, pulmonary alveolar, and splenic macrophages were not reduced⁵⁹⁻⁶². These results provide evidence that tissue macrophages can survive for a long time by self-renewal, without a supply of blood monocytes. Similar monocytopenic mice were produced by a technique providing fractional radiation to bone marrow alone, with shielding of the other organs and tissues⁶³. In these monocytopenic mice, pulmonary alveolar macrophages survived by self-renewal⁶³. We also examined the proliferative capacity of Kupffer cells in the liver of ⁸⁹Sr-treated, severely monocytopenic mice and confirmed that Kupffer cells slightly increased in number with time after injection and that their proliferative capacity also increased, in parallel with their numerical increase⁶⁴. All these data clearly show that macrophages have a proliferative capacity and can survive for a long time by self-renewal, without a supply of blood monocytes.

3) Relationship between monocytes and tissue macrophages

In response to inflammatory stimuli, monocytes are prominently produced in and mobilized from the bone marrow, migrate into

inflammatory lesions via the blood stream, and differentiate into exudate macrophages *in loco*. Although many factors participate in monocyte migration, monocyte chemoattractant protein-1 (MCP-1) is the most powerful factor for induction of monocyte infiltration into inflammatory foci^{65,66}. However, MCP-1 has no effect on induction of migration and infiltration of tissue macrophages into inflammatory foci. Therefore, monocytes are the important cells in inflammatory foci and respond to the MCP-1 produced by many types of cells, such as vascular endothelial cells, fibroblasts, tissue macrophages, and T lymphocytes⁶⁶.

To investigate the relationship between monocytes and tissue macrophages, we produced prominent monocytosis in mice by daily intravenous injections of macrophage colony-stimulating factor (M-CSF)⁶⁷ or in mice with the use of subcutaneous transplants of M-CSF- and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing mouse fibrosarcoma⁶⁸. We examined changes in the number of tissue macrophages in various organs and tissues of these mice. We found that the organs and tissues are not stimulated in these monocytic mice: the numbers of tissue macrophages are not increased^{36,67,68}. It has recently become clear that

monocytes circulating in peripheral blood undergo apoptosis under unstimulated conditions and do not enter peripheral tissues⁶⁹. In transgenic mice producing an excess of GM-CSF, peritoneal macrophages are sustained by self-renewal, independent of blood monocytes⁷⁰. These data provide evidence that there is no relationship between monocytes and tissue macrophages under unstimulated conditions.

4) Life span of macrophages

According to the MPS concept, macrophages are terminal cells of the MPS; they lose proliferative capacity and are supplied from blood monocytes^{5,18-20}. In studies with [³H] thymidine autoradiography, van Furth and co-workers determined that the turnover times for Kupffer cells, splenic or alveolar macrophages, and peritoneal macrophages were 3.8 days, 6 days, and 14.9 days, respectively^{18-20,71}. Thus, monocyte-derived macrophages are short-lived in peripheral tissues and die within 2 weeks.

In contrast, tissue macrophages can survive by self-renewal for long periods⁵⁹⁻⁶⁴. Previous studies have presented variable data about their life span; however, these studies did show that tissue macrophages have a long life span. In mice, de Bakker and Daems, using Imferon (iron

Table 4. Macrophages in invertebrates and vertebrates

Animals	Macrophages (phagocytic Cells)
Invertebrates	
Unicellular animals	
<i>Protozoa</i> (ameba, paramecia)	Animals themselves are the primitive form of macrophages
Didermic animals	
<i>Porifera</i>	Archeocytes
<i>Coelenterata</i> (jelly fish, coral, sea anemone)	Amebocytes
Tridermic Protostomia	
<i>Annelida</i> (earthworm, leech, nereid)	Coelomocytes
<i>Mollusca</i> (Incilaria, octopus, squid, clam, oyster)	Ameboid cells (macrophages)
<i>Arthropoda</i>	
<i>Insecta</i> (<i>Spodoptera litura</i>)	Granular or ameboid plasmatocytes
<i>Crustacea</i> (prawn, shrimp, scrab, crayfish)	Granular cells, semi-granular cells, hyaline cells
Tridermic Deuterostoma	
<i>Echinodermata</i> (starfish, echinoid, sea urchin)	Phagocytes (phagocytic amebocytes)
<i>Protochordata</i> (Botryllus, Amphioxus)	Small granular amebocytes
Vertebrates	
<i>Cyclostomes</i> (lamprey, hagfish)	Macrophages (gills, liver), monocytes
Cartilaginous fish (shark, ray)	Macrophages (gills, spleen, liver, kidneys), monocytes
Teleost fish (goldfish, cyprinoid)	Macrophages (gills, spleen, kidneys, heart, peritoneal cavity), monocytes
<i>Amphibia</i> (frog, newt, salamander)	Macrophages (liver, spleen, kidneys, peritoneal cavity, bone marrow, jugular body), monocytes
<i>Reptilia</i> (lizard, snake, tortoise)	Macrophages (ubiquitously distributed), monocytes
<i>Aves</i> (pigeon, fowl, goose)	Macrophages (ubiquitously distributed), monocytes
<i>Mammalia</i> (mouse, rat, guinea pig, monkey, human)	Macrophages (ubiquitously distributed), monocytes

dextran) as a lysosomal marker, reported that peritoneal resident macrophages can survive for 4 months⁷², and Melnicoff *et al.* in their studies with a fluorescent dye PKH-1 demonstrated that PKH-1-labeled peritoneal resident macrophages are detected at 49 days after labeling⁷³. For Kupffer cells, different data were presented. In contrast with data presented for mice by van Furth *et al.* that the life span of Kupffer cells is about 4 days⁷¹, other investigators reported a longer life span, 100 days or about 14 months, in rats^{74,75}. We demonstrated in a recent study of mouse liver by flash labeling with [³H] thymidine that the numbers of labeled Kupffer cells were maintained above the baseline for 5 weeks after labeling, which indicates that the life span of Kupffer cells is about 5 weeks⁷⁶. In summary, these data show that tissue macrophages are long-lived and can survive for long periods.

II. Phylogeny of macrophages

Macrophages are present in tissues in all multicellular invertebrates and vertebrates (Table 4). *Protozoa* are unicellular animals that take up nutrients or pathogenic microorganisms by phagocytosis, secrete various substances including enzymes, and protect themselves against microorganisms^{77,78}. Because these functions are common to those of macrophages, *Protozoa* are considered to be a prototype of macrophages. During the evolution from unicellular animals to multicellular ones, professional phagocytic cells developed in tissues; these cells have been given different names. In bidermic animals such as *Porifera*, archeocytes are present in a mesoglea between the epithelial cells of the external body surfaces and the internal choanocytes, and they ingest, digest, and degrade various materials and dead cells⁷⁹. *Coelenterata* such as sea anemones or jellyfish have wandering ameboid cells, which are called amebocytes and possess phagocytic functions⁸⁰. The archeocytes in *Porifera* and the amebocytes in *Coelenterata* are regarded as primitive forms of macrophages that exist in multicellular animals; these cells are totipotent stem cells, which can differentiate into various types of cells. In *Hydra*, however, there are no cells corresponding to archeocytes in *Porifera*, amebocytes in *Coelenterata*, or macrophages in higher multicellular animals; instead, endoder-

mal epithelial cells show phagocytic functions⁸¹.

Tridermic invertebrates develop, besides the endoderm and the ectoderm, a mesoderm, from which ameboid phagocytic cells develop. In *Platyhelminthes*, planarian reticular cells are macrophage-like cells that have phagocytic functions and encapsulate heat-killed human tuberculosis bacilli⁸². As shown in Table 4, mononuclear phagocytes or macrophage-like cells have different names according to the species of animal in which they occur. In invertebrates, monocytes are not detected in blood. However, monocytes are observed in the blood of cyclostomes such as lampreys and hagfish, the most primitive vertebrates, and macrophages are seen in gills and spleen⁸³. From cartilaginous fish to mammals, resident macrophages are found in various tissues, and monocytes are detected in peripheral blood. Although bone marrow is not developed in cyclostomes, cartilaginous fish, or teleost fish, macrophages do exist in tissues⁸³⁻⁸⁵. In *Amphibia*, bone marrow develops in frogs but not in salamanders. In frogs, tissue macrophages involved in phagocytosis of apoptotic muscle cells during tail involution are considered not to be derived from monocytes⁸⁶. In *Reptiles*⁸⁷, *Aves*⁸⁸, and *Mammalia*, macrophages have a ubiquitous distribution in various tissues.

In terms of phylogeny, therefore, unicellular animals are believed to be a primitive form of macrophages; professional phagocytes (macrophages) develop in multicellular invertebrates, followed by the emergence of monocytes in vertebrates from cyclostomes to mammals, and the development of bone marrow in *Reptiles*, *Aves*, and *Mammalia* (Table 4). This information suggests that during the evolutionary processes in animals, macrophages develop before monocytes emerge, which conflicts with the basic concept of MPS, that all macrophages are derived from monocytes.

III. Ontogenesis of macrophages and related cells

1. Ontogeny of Macrophages

The phylogenetic principle that macrophage development occurs before the emergence of monocytes is seen in the ontogeny of mammals. Whereas in postnatal and adult life in normal animals and humans, hematopoiesis occurs exclu-

sively in the bone marrow, in human, rat, and mouse fetuses, primitive fetal hematopoiesis starts in the yolk sac, and, in line with ontogeny, progresses to the fetal liver and finally to the bone marrow. At the stage of progression to the liver, permanent or definitive hematopoiesis develops in the gonads, around the aorta and in the mesonephros (called the GAM area) and occurs in the fetal liver, gradually replacing primitive hematopoiesis⁸⁹.

In primitive hematopoiesis in the yolk sac in human embryos, macrophages make up approximately 70% of the total nucleated blood cells at 4 weeks⁹⁰. In mouse and rat embryos, macrophages predominate in the early stage of primitive hematopoiesis in the yolk sac; monocytic cells are absent⁹¹⁻⁹³. We observed immature and mature macrophages in the yolk sac by light and electron microscopy; we called the immature macrophages "primitive macrophages" and the mature macrophages "fetal macrophages"^{91,92}.

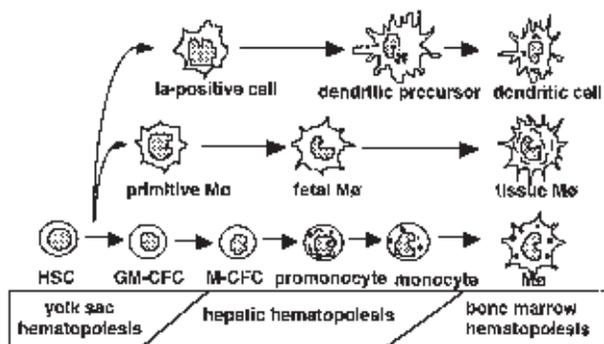


Fig. 2. Ontogenetic development, differentiation, and maturation of macrophages and dendritic cells (HSC: hematopoietic stem cell, GM-CFC: granulocyte/macrophage colony-forming cell, M-CFC: macrophage colony-forming cell, Mφ: macrophage).

The present author maintained that primitive macrophages are differentiated from hematopoietic stem cells, bypassing the developmental stage of monocytic cells, and mature into fetal macrophages (Fig. 2). The hematopoietic transcription factor PU. 1, which determines the differentiation of hematopoietic stem cells into macrophages and B lymphocytes, is expressed in blood cells of the fetal liver and bone marrow^{94,95}. In the early stage of yolk sac hematopoiesis, however, PU. 1 is not expressed in any type of hematopoietic cells, although many primitive and fetal macrophages have already developed^{94,95} (Table 5). These data provide evidence that the primitive and fetal macrophages are derived from early PU. 1-negative hematopoietic progenitor cells and not from monocytic cells^{94,95} (Table 5). The primitive and fetal macrophages possess a high proliferative capacity and their [³H] thymidine labeling rate is approximately 60-70% in the murine yolk sac^{91,92}.

After the embryo yolk sac connects with the cardiovascular system via vitelline veins, primitive and fetal macrophages migrate from the yolk sac into various organs and tissues, particularly the fetal liver, proliferate in these organs and tissues, and expand their population *in loco*⁹⁶. Migration of primitive and fetal macrophages from the yolk sac into tissues occurs at 10 fetal days in mice and at 11 fetal days in rats. In the murine fetal liver, primitive hematopoiesis occurs at about 12 fetal days, and numerous primitive and fetal macrophages develop, actively proliferate, and differentiate into Kupffer cells⁹⁶⁻⁹⁹. In murine fetal lungs, macrophages are derived from macrophage precursors before the monocytic cell stage¹⁰⁰⁻¹⁰³, proliferate, and differentiate into pulmonary interstitial macrophages

Table 5. Classification of macrophages and related cells in mutant mice

PU. 1-independent macrophages Primitive and fetal macrophages in primitive fetal hematopoiesis Large tissue macrophages develop in long-lived PU. 1 ^{-/-} mice by administration of antibiotics
PU. 1-dependent macrophages (absent in PU. 1 ^{-/-} mice) <ol style="list-style-type: none"> 1) M-CSF-independent macrophages (develop in <i>op/op</i> mice) Immature tissue macrophages: splenic red pulp, lungs, perivascular regions of brain, microglia (hippocampal areas) 2) M-CSF-dependent macrophages and related cells (absent in <i>op/op</i> mice) <ol style="list-style-type: none"> a) Rapidly M-CSF-responding population M-CFCs, monocytic cells, monocyte-derived macrophages, osteoclasts b) Non- or slowly M-CSF-responding population Marginal zone macrophages and marginal metallophilic macrophages in spleen, synovial A cells, macrophages in ovaries, uterus, and renal interstitium

and further into alveolar macrophages after birth¹⁰⁴. In the other fetal organs and tissues, primitive and fetal macrophages proliferate and differentiate into tissue-specific macrophages¹⁰⁵⁻¹⁰⁸. As gestation progresses, the proliferative capacity of macrophages declines in most fetal organs and tissues³⁶.

In the liver of human, rat, and mouse fetuses, primitive hematopoiesis originating in blood islands of the yolk sac is replaced by permanent hematopoiesis from the GAM area⁸⁸. In the fetal mouse liver, monocytic cells start to develop at 14 fetal days and increase their numbers by proliferation of monoblasts and promonocytes¹⁰⁹. At about 18 fetal days, monocytes are detected in the peripheral circulation¹¹⁰, migrate into tissues, and differentiate into monocyte-derived macrophages³⁶. In the early stage of ontogeny, primitive and fetal macrophages circulate in peripheral blood; however, their numbers are reduced in parallel with the progression of gestation, and they finally disappear at birth. In contrast, monocytes rather than primitive and fetal macrophages are released from fetal hematopoietic organs in the late stage of ontogeny, circulate in peripheral blood, and migrate into peripheral fetal organs and tissues to become monocyte-derived macrophages³⁶.

Primitive and fetal macrophages are thus different from monocyte/macrophages in terms of the stage of emergence, the site of development, and pathways of differentiation (Fig. 2).

2. Ontogeny of dendritic cells

In human yolk sac hematopoiesis, dendritic cell precursors develop distinctly and independently from macrophage precursors¹¹¹. In murine ontogeny, however, our studies have revealed that dendritic cell precursors are similar to the precursors of primitive and fetal macrophages, express Ia antigens in various fetal tissues at 12 fetal days, and become Ia-positive macrophages³⁶. At 14 fetal days, Ia-positive macrophages migrate into the thymic primordium to induce Ia expression of thymic epithelial cells, which occurs at 15 fetal days¹¹²⁻¹¹⁴. These Ia-positive macrophages show a dendritic cell morphology, differentiate into interdigitating cells, and develop tubulovesicular structures in the cytoplasm at 16 fetal days¹¹²⁻¹¹⁴. Tubulovesicular structures are localized around

the Golgi complex area of interdigitating cells, consisting of many vesicles, tubules and granules. After birth, Birbeck granules develop in the cytoplasm of some interdigitating cells and a minor population of Langerhans cells appears in the thymic medulla^{112,113}.

At 12 fetal days in the rat, primitive and fetal macrophages emerge in the subepidermal mesenchyme; some of them express Ia antigens at 14 fetal days, and they migrate into the epidermis at 16 fetal days^{115,116}. From 18 fetal days on, these cells start to show a dendritic cell morphology and differentiate into interdigitating cells in the epidermis^{115,116}. In the perinatal period, the interdigitating cells further develop Birbeck granules in the cytoplasm and differentiate into Langerhans cells^{115,116}. The proliferative potential of the epidermal dendritic cells, which is about 10-20% in the late fetal stage and 5% after birth, is important for the expansion and maintenance of Langerhans cells in the epidermis during perinatal, postnatal, and adult life¹¹⁶.

In the fetal rat spleen, Ia-positive macrophages initially appear around the central arteries in splenic lymphoid follicles at 16 fetal days and start extending dendritic processes¹⁰⁵. With the advance of gestation, the numbers of dendritic cells increase, and the cells are localized in the innermost layer of the periarteriolar lymphoid sheaths¹⁰⁵. In fetal lymph nodes, Ia-positive macrophages appear around blood vessels in the paracortical areas from 17 fetal days on and thereafter differentiate into interdigitating cells³⁶.

In murine ontogeny, Ia-positive macrophages are derived from hematopoietic stem cells via primitive macrophages, differentiate into interdigitating cells, and mature into Langerhans cells in the epidermis and thymic medulla after birth (Fig. 2).

IV. Development and differentiation of macrophages in postnatal and adult life

In normal steady-state conditions, macrophages are terminal cells differentiating from hematopoietic stem cells in bone marrow. The bone marrow possesses specific mechanisms to release and mobilize macrophage progenitor cells into the peripheral circulation, and there are two pathways for supplying macrophage precursors. When hematopoietic stem cells in the bone

marrow enter a stage of lineage-specific differentiation, they are not released from the bone marrow unless they differentiate into monocytes via monoblasts and promonocytes^{5,16-20,117}. During differentiation, monocytes obtain PO-positive cytoplasmic granules and lose their proliferative capacity. In addition, a small number of immature macrophage progenitor cells, at the pre-monocytic cell lineage stage of differentiation, are released from the bone marrow into peripheral blood, migrate into peripheral tissues, and differentiate into tissue macrophages^{36,117}. Tissue macrophages have a proliferative capacity, and their [³H] thymidine labeling rate remains at approximately 2-5% through their life span^{5,18,19}.

In the following sections, the differentiation and maturation of monocyte-derived macrophages and tissue macrophages are discussed. In addition, a differentiation pathway of common lymphoid progenitors into B cell precursors and further into B cell-derived macrophages is described.

1. Differentiation and maturation of monocyte/macrophages

On the basis mainly of the results of studies by van Furth and co-workers, it has been established that hematopoietic stem cells divide several times and become monoblasts^{5,18-20,118}. One monoblast divides once into two promonocytes, and one promonocyte also divides once into two monocytes. In this differentiation pathway, one monoblast becomes four monocytes^{5,18-20}. In stimulated conditions, monocytes adhere to activated vascular endothelial cells, migrate into tissues, and differentiate into exudate macrophages⁶⁹. The development of monocytic cells (monoblasts, promonocytes, and monocytes) and the differentiation of monocytes into macrophages are supported by M-CSF¹¹⁹.

Mice with an osteopetrosis (*op*) mutation, *op/op* mice, show a total lack of M-CSF activity because of a defect in the coding region of the *c-fms* gene; show an absence of incisors, a distinctly domed skull, a short tail, and a small body at about 10 days after birth; and have a defect in bone remodeling caused by a complete deficiency of functional osteoclasts, leading to systemic osteosclerosis in skeletal bones, prominent stricture of bone marrow cavities, and marked reduction of medullary hematopoiesis¹¹⁹. In addition,

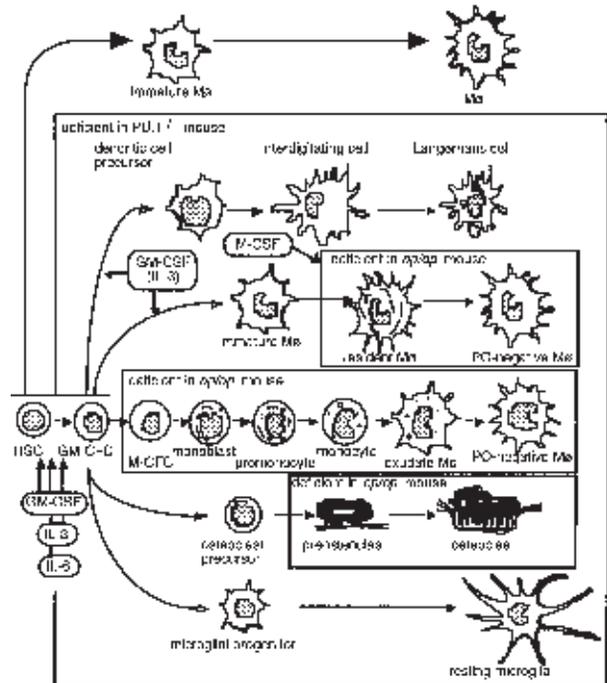


Fig. 3. Development, differentiation, and maturation of macrophages and related cells, including dendritic cells, in adult life (HSC: hematopoietic stem cell, GM-CFC: granulocyte/macrophage colony-forming cell, M-CFC: macrophage colony-forming cell, M ϕ : macrophage).

op/op mice show a complete or nearly complete deficiency of monocytes in peripheral blood, severe impairment in production of monocytic cells and their precursors in bone marrow, complete deficiency of monocyte-derived macrophages, and defective differentiation of monocytes into macrophages in tissues¹¹⁹. Daily M-CSF administration to *op/op* mice induces a marked increase in the number of blood monocytes, production of monocytic cells in the bone marrow, and differentiation and maturation of monocyte-derived macrophages in tissues and of osteoclasts in bone¹²⁰. In the bones of *op/op* mice, osteoclasts start to develop after daily M-CSF administration, and the number of these cells increases up to the level found in normal littermates at 3 days after administration¹²⁰. Therefore, osteoclasts and monocyte-derived macrophages are termed the "M-CSF-dependent population"³⁶ (Table 5). This cell population responds rapidly to M-CSF and are supported by M-CSF. In *op/op* mice, marginal metallophilic macrophages and marginal zone macrophages in the spleen are absent, and synovial A cells,

macrophages in uterus and ovaries, and microglia in the brain are few, compared with wild-type mice^{120,121}. Because these cells do not appear or develop slowly after daily M-CSF administration^{120,121}, they are called the nonresponding or slowly responding population; their development may depend on M-CSF produced *in situ*³⁶ (Table 5). M-CSF supports the proliferation and differentiation of monocytic cells and their precursors (macrophage colony-forming cells; M-CFCs), so all of these macrophage populations are derived from M-CFCs and/or monocytic cells^{36,120} (Fig. 3).

Although various chemotactic factors such as MCP-1, MCP-2, MCP-3, MCP-4, M-CSF, GM-CSF, tumor necrosis factor- α (TNF- α), transforming growth factor- β (TGF- β), and RANTES (regulated on activation, normal T cell expressed and secreted) are known to induce chemotaxis of monocytes and macrophages into tissues, MCP-1 is the most potent CC chemokine responsible for infiltration of monocytes into tissues^{65,66}. In pathological conditions, when inflammatory stimuli occur in tissues, vascular endothelial cells, fibroblasts, tissue macrophages, infiltrated monocyte/macrophages, and T cells produce MCP-1 to induce monocyte infiltration into the tissues^{122,123}. In unstimulated normal tissues, MCP-1 is not expressed, and monocytes continue to circulate in peripheral blood and die by apoptosis without infiltration into tissues. MCP-1 does not induce migration and infiltration of any tissue macrophages into inflammatory lesions^{122,123}.

2. Differentiation and maturation of tissue macrophages

In contrast to monocyte/macrophages and their related macrophage populations, tissue macrophages develop in various organs and tissues of *op/op* mice¹¹⁹. These cells are small, round, and immature, with an ultrastructure characterized by poor development of intracellular organelles, particularly lysosomal granules, and they show no cytochemical localization of PO activity¹¹⁹. These immature tissue macrophages are present in various organs and tissues of *op/op* mice, particularly in the lungs, spleen, and brain; their numbers are reduced in other tissues to a variable degree¹¹⁹. Because *op/op* mice lack functional activity of M-CSF and

monocytic cells in peripheral blood, the immature tissue macrophages are called "M-CSF-independent macrophages" (Table 5) and are considered to be derived from earlier macrophage precursor cells than monocytic cells and M-CFCs^{36,119,120}.

As mentioned above, bone marrow releases macrophage precursor cells at the stage before lineage-specific differentiation of hematopoietic cells, and immature macrophage precursor cells migrate into tissues in response to various chemotactic factors. Pre-B cell growth-stimulating factor (PBSF)/stromal-derived factor (SDF)-1 is one of the CXC chemokines; it stimulates proliferation of B cell progenitors *in vitro* and is constitutively expressed in bone marrow-derived stromal cells^{124,125}. Mice lacking PBSF/SDF-1 or CXC receptors die in the perinatal stage of development, the number of B cell progenitors is severely reduced in fetal liver and bone marrow of mutant embryos, and myeloid progenitor numbers are reduced only in the bone marrow, not in the fetal liver¹²⁶⁻¹²⁸. Thus, PBSF/SDF-1 is responsible for B cell lymphopoiesis and bone marrow hematopoiesis and can induce migration of early hematopoietic progenitors into tissues. In normal mice, PBSF/SDF-1 mRNA is expressed in all organs and tissues except for the epidermis, which suggests that these organs and tissues can induce the migration of early hematopoietic progenitors by the action of PBSF/SDF-1.

After migration of early hematopoietic progenitors into tissues, these cells can differentiate into tissue-specific macrophages in response to interleukin (IL)-6, IL-3, GM-CSF, M-CSF, and other cytokines produced *in situ*. In *op/op* mice, in spite of the absence of blood monocytes, immature macrophages differentiate from early hematopoietic progenitors without the action of M-CSF in various organs and tissues. Immediately after M-CSF administration, these immature macrophages proliferate and express PO activity in the nuclear envelope and rough endoplasmic reticulum, a finding that is consistent with the localization pattern of PO activity in resident macrophages¹²⁹. Thus, resident macrophages in tissues are considered to be differentiated from earlier myeloid precursors in tissues; they bypass the stage of M-CFCs and monocytic cells.

Although various transcription factors are known to be involved in the development and

differentiation of hematopoietic stem cells into tissue macrophages, PU. 1 is a critical hematopoietic transcription factor for the differentiation of early hematopoietic progenitors into macrophages and B cells¹³⁰. In normal mice, PU. 1 mRNA is expressed in all organs and tissues. PU. 1-deficient mice usually die in the fetal stages or die of septicemia within 2 days after birth^{131,132}. In these mutant fetuses or neonatal mice, all macrophages, including not only monocytic cells and monocyte-derived macrophages but also tissue macrophages, are completely absent^{131,132}. In the mutant embryos and fetuses, macrophages are absent in the yolk sac, fetal liver, and bone marrow^{131,132}. Hematopoietic progenitor cells from PU. 1^{-/-} mice do not respond to GM-CSF or M-CSF¹³³. However, when mutant mice are rescued by treatment with antibiotics immediately after birth and survive for 2 weeks, a small number of large macrophages develop in various tissues such as the liver and bone marrow¹³¹. This result suggests that tissue macrophages can develop from early hematopoietic progenitor cells in PU. 1^{-/-} mice (Table 5) and that the development and differentiation of early hematopoietic progenitors into tissue macrophages occur not only in early ontogeny but also in postnatal life (Fig. 3).

3. Development and differentiation of B lymphoid progenitors into macrophages

As shown in PU. 1- or PBSF/SDF-1-deficient mice, macrophages and B cells are related to each other at the gene level^{126-128,130-132}. B cells are classified into two major subsets on the basis of expression of CD5 (Ly-1): CD5-positive B cells (B-1) and CD5-negative B cells (B-2)^{134,135}. B-2 cells are conventional B lymphocytes; B-1 cells are derived and differentiated from precursor cells in the omental milky spots, proliferate by self-renewal in the peritoneal cavity, and produce natural antibodies and autoantibodies^{135,136}. One subset of B-1 cells continues to express CD5 (B-1a cells), whereas the other subset loses CD5 during maturation (B-1b cells)¹³⁷. *In vitro* studies of pre-B cell lines or myeloid cell lines introduced by *v-raf* and/or *v-myc* genes revealed that B cells differentiate into macrophages^{138,139}. In our previous studies of pre-B cell lines established in a long-term bone marrow culture, we confirmed *in vitro* that a

pre-B cell line, J13, differentiates into CD5-positive macrophages at 1 month after co-culture with GM-CSF and a mouse bone marrow stromal cell line, ST2^{140,141}. We examined many different mouse strains including immunodeficient mice such as nude, severe combined immunodeficiency (scid), X-linked immunodeficiency (xid), and viable motheaten (*me^v/me^v*) mice to detect CD5-positive macrophages. In all these mice except for *me^v/me^v* mice, we did not detect CD5-positive macrophages as a cell population; however, we did find many CD5-positive macrophages in the peritoneal cavity, spleen, and lymph nodes of *me^v/me^v* mice¹⁴².

The *me^v/me^v* mice show tyrosine phosphatase deficiency in hematopoietic cells because of mutations in the SHP-1 gene, which results in abnormal signal transduction in hematopoietic cells and induces severe impairments of T and B cell differentiation, a severe reduction in the numbers of B-2 cells, increased numbers of B-1 cells with abnormal immunoglobulin production, enhanced production of myeloid cells, and increased production of monocyte/macrophages in bone marrow and their infiltration into various organs and tissues¹⁴². In *me^v/me^v* mice, the concentration of GM-CSF is elevated in serum and peritoneal fluid, suggesting that GM-CSF induces the development and differentiation of CD5-positive macrophages¹⁴².

To verify this suggestion, we injected BALB/c or C3H mice intravenously with 5 ng of GM-CSF for 5 days¹⁴¹. We found the emergence of numerous CD5-positive macrophages in the peritoneal cavity of the GM-CSF-treated mice but not in the untreated mice¹⁴¹. Besides the CD5-positive peritoneal macrophages, we found precursor cells of both B cells and CD5-positive macrophages or CD5-positive progenitor cells¹⁴¹. The presence of such common precursor cells for both B-1 cells and macrophages implies that CD5-positive macrophages are derived from common B lymphoid progenitors. However, daily administration of GM-CSF to scid, xid, *aly/aly* mice failed to produce CD5-positive macrophages in the peritoneal cavity, because the scid and xid mice showed severe impairments in B cell development and differentiation, and the *aly/aly* mice had a severe deficiency of lymphoid tissues^{141,143}. In addition, daily administration of GM-CSF to M-CSF-deficient *op/op* mice did not induce the development of CD5-positive macrophages in the

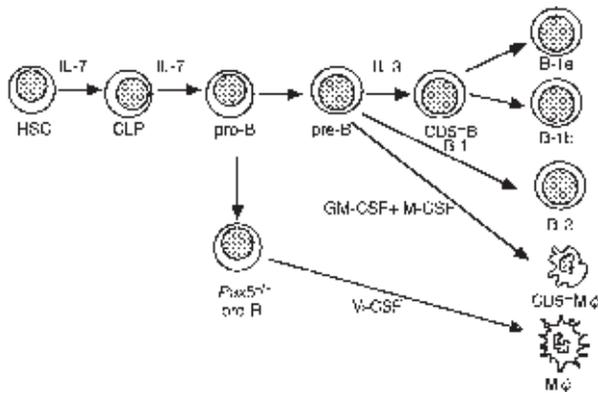


Fig. 4. Schematic diagram of the differentiation of common lymphoid progenitors into macrophages (HSC: hematopoietic stem cell, CLP: common lymphoid progenitor, M ϕ : macrophage).

peritoneal cavity, indicating that M-CSF is also essential for the development of CD5-positive macrophages¹⁴¹. These data indicate that both hematogenous administration of GM-CSF and endogenous production of M-CSF are important for the development and differentiation of CD5-positive macrophages. In peritoneal cells of untreated BALB/c mice, PU. 1 mRNA was not detected; however, it was expressed in these mice after daily administration of GM-CSF, as well as in *me^v/me^v* mice¹⁴³. This result shows that GM-CSF induces PU. 1 mRNA for development and differentiation of CD5-positive macrophages. The CD5-positive macrophages are called biphenotypic B/macrophage cells, indicating a link between B cells and macrophages^{144,145}.

In scid mice, omental milky spots are absent, and neither CD5-positive macrophages nor B-1 cells are detected in the peritoneal cavity¹³⁷. According to the method of Solvason *et al*¹³⁷, we transplanted fetal murine liver cells and adult bone marrow cells into scid mice. At 1 month after transplantation of C3H mouse fetal liver or adult bone marrow into the renal capsule of scid mice, daily administration of GM-CSF for 5 days induced the development of CD5-positive macrophages in the peritoneal cavity¹⁴⁶. CD5.1 recognizes B-1 cells of donor C3H mice, whereas CD5.2 reacts with those of recipient scid mice. By using both monoclonal antibodies, we could demonstrate that CD5-positive macrophages that emerged in the peritoneal cavity after daily administration of GM-CSF showed reactivity for CD5.1, indicating that the macrophages are der-

ived from hematopoietic progenitors originating in the fetal liver or bone marrow of the donor C3H mice¹⁴⁶. Numerous CD5.1-positive macrophages and their precursors were also observed in the milky spots developing in the scid mice¹⁴⁶. These data suggest that B lymphoid progenitors are derived from fetal liver or adult bone marrow, migrate into omental milky spots, and differentiate into CD5-positive macrophages. CD5-positive macrophages that develop in the omental milky spots move to and appear in the peritoneal cavity. In addition, bipotential precursors for B cells and macrophages have been demonstrated in murine fetal liver¹⁴⁷.

Early B cell lymphogenesis is regulated by at least three transcription factors: basic helix-loop-helix proteins encoded by the *E2A* gene¹⁴⁸, early B cell factor¹⁴⁹, and B cell-specific activator protein, which is encoded by the *Pax5* gene and recognizes DNA through the highly conserved paired domain characteristic of the *Pax* family of transcription factors¹⁵⁰. The *Pax5* gene is expressed exclusively in the B lymphoid lineage and is required *in vivo* for propagation beyond the pro-B cell stage. In *Pax5*-deficient mice, pro-B cells are incapable of *in vitro* B cell differentiation, are not restricted to the fate of their lineage, and differentiate into macrophages after stimulation with M-CSF or into related cells such as osteoclasts or dendritic cells after supplementation with other appropriate cytokines *in vitro*¹⁵⁰. On the basis of all this information, it appears obvious that B lymphoid progenitors can differentiate into macrophages and their related cells *in vivo* and *in vitro* (Fig. 4).

4. Development and differentiation of macrophage-related cells

1) Osteoclasts

Because various types of osteopetrosis are known to occur in humans and animals as a result of defects of osteoclasts or in their functional abnormalities, it is important for the elucidation of osteoclast development, differentiation, and biology to analyze these human and animal osteopetrotic diseases. Osteoclasts are multinuclear cells positive for tartrate-resistant acid phosphatase (TRAP) stain, have brush borders facing bone matrix, and are formed by fusion of TRAP-positive mononuclear cells (preosteoclasts). In *op/op* mice, a defect in development of

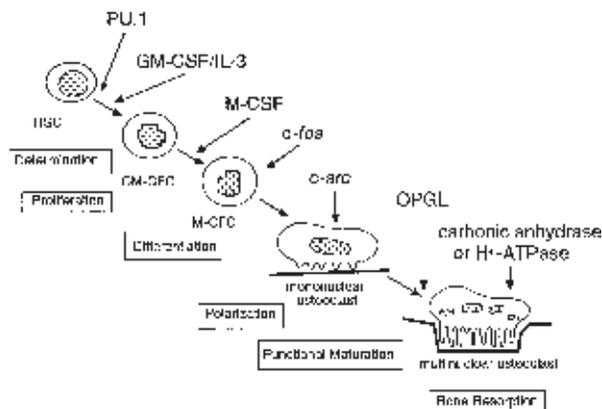


Fig. 5. Schematic diagram of osteoclast development, differentiation, and maturation (HSC: hematopoietic stem cell, GM-CFC: granulocyte/macrophage colony-forming cell, M-CFC: macrophage colony-forming cell).

osteoclasts and monocytic cells from hematopoietic stem cells via granulocyte/macrophage colony-forming cells (GM-CFCs) results from a genetic defect in the production of a functional M-CSF protein, leading to the occurrence of osteopetrosis^{119,120}. Daily administration of M-CSF induces proliferation of GM-CFCs and their differentiation into osteoclasts, bypassing the monocytic cell stage^{120,151}. In aged *op/op* mice, GM-CSF and IL-3 levels are increased in serum, which recover osteopetrosis¹⁵². In young *op/op* mice, osteopetrosis is improved by daily administration of GM-CSF or IL-3 or both¹⁵². In M-CSF-, GM-CSF- or IL-3-treated *op/op* mice, the fusion of preosteoclasts into osteoclasts is evidently observed^{120,151,152}.

In PU. 1-deficient mice, osteopetrosis occurs as a result of defects in development of osteoclasts, monocyte-derived and tissue macrophages, dendritic cells, and B cells^{131,153,154}. *c-fos* forms heterodimers of Jun protein and AP-1, regulates various transcription factors, and is involved in cell proliferation. In *c-fos*-deficient mice, the differentiation of hematopoietic stem cells into osteoclasts is blocked, leading to the occurrence of osteopetrosis¹⁵⁵; however, differentiation of these cells into macrophages is not impaired¹⁵⁵. In *c-src*-deficient mice, osteoclasts lack brush borders, which are indispensable for bone resorption; however, the differentiation of hematopoietic stem cells into osteoclasts is not impaired¹⁵⁶.

OPGL (osteoprotegerin ligand), which

belongs to a TNF family called ODF (osteoclast differentiation factor), TRANCE (TNF-related activation-induced cytokine), or RANKL (receptor-activator of NF- κ B ligand), activates the formation of multinuclear osteoclasts in the presence of M-CSF, and is a regulating factor for interactions with T cells and dendritic cells¹⁵⁷. In OPGL-deficient mice, severe osteopetrosis develops because of a complete defect of osteoclasts resulting from a functional abnormality of osteoblasts so that they do not support the formation of osteoclasts¹⁵⁷. Mature osteoclasts have a proton pump and an enzyme, carbonic anhydrase II, specific for bone resorption^{158,159}. In humans, it is known that osteopetrosis occurs because of a deficiency in H⁺-ATPase¹⁵⁸ or carbonic anhydrase II¹⁵⁹.

It is thus concluded that osteoclasts develop from hematopoietic stem cells and differentiate via GM-CFCs, bypassing the differentiation stage of monocytic cells, and become polarized, forming brush borders (Figs. 3 and 5).

2) Microglial cells

Microglia were first described by Del Rio-Hortega¹⁶⁰ as resting microglia, a population of cells residing in the central nervous system under a normal steady state. Since then, many cells have been proposed for the origin of microglia, including mesenchymal cells, perivascular cells (pericytes), monocytes, glial cells, and neural cells¹⁶¹. Fujita and Kitamura in Japan concluded from electron microscopic studies with [³H] thymidine autoradiography that microglia develop in the brain after birth and are derived from glioblasts, the same progenitor as that for astrocytes and oligodendrocytes¹⁶². However, recent studies using monoclonal antibodies against macrophages have shown that resting microglia share a common phenotype with macrophages, providing evidence that the cells are related to macrophages¹⁶³⁻¹⁶⁵. On the basis of differences in cell morphology, ultrastructure, developmental stage, and precursor cells, microglia are classified into three subtypes: 1) ameboid microglia, 2) resting microglia, and 3) reactive microglia¹⁶¹. Ameboid microglia are found in the embryonic and fetal stages, emerge in fetal rat brain at 12 fetal days, and enter, along with the extension of blood vessels from the meninges, the fetal brain parenchyma. Ameboid microglia are differentiated from a subpopulation of primitive

and fetal macrophages derived from hematopoietic stem cells in yolk sac primitive hematopoiesis¹⁶⁶. The microglia have a high proliferative capacity and are distributed in the fetal rat brain¹⁶⁵. In the late stage of ontogeny, they move into the soft meninges, the perivascular areas, and the choroid plexus, around the brain ventricles¹⁶⁵, and to the deeper areas of fetal brain parenchyma^{167,168}. From the late stage of ontogeny to the early neonatal period, because ameboid microglia move to the deeper regions of the brain parenchyma, they disappear in the brain cortex and subcortical regions. However, these microglia start to proliferate as progenitors of resting microglia in the neonatal rat brain and move to the brain cortex and subcortical regions¹⁶⁹, and resting microglia distribute in the whole rat brain and appear at 4 weeks after birth¹⁶⁶.

In *op/op* mice, the number of resting microglia in the brain is not reduced in the hippocampal area, compared with the number of cells in the brain of wild-type mice^{170,171}. The resting microglia are an M-CSF-independent cell population and are differentiated from microglial progenitor cells at the stage before monocytic cell series, presumably GM-CFCs¹⁷¹. However, our studies showed variable reductions in the numbers of microglia in areas other than the hippocampus in *op/op* mice^{36,120}. This population of microglia did not recover after daily administration of M-CSF^{36,120}. They are supported, however, by growth factors such as GM-CSF and IL-3. Our study with daily intravenous injections of murine GM-CSF or IL-3 into *op/op* mice showed that F4/80-positive cells increase in number in brain tissues and that their shape is small, round, and not ramified (data not published). Therefore, certain factors other than GM-CSF and IL-3 may be necessary for the differentiation of microglial progenitor cells into resting microglia. Studies with intravenous injections of cells obtained from primary cultures of microglia or immortalized microglial cells demonstrated that these cells enter the brain parenchyma through the walls of blood vessels, move into brain tissues, and change into ramified resting microglia¹⁷²⁻¹⁷⁴.

Reactive microglia are usually found in inflammatory or damaged brain tissues and are derived from monocytes invading *in loco*. Experimental studies with intravenous injections of isolated microglia, blood monocytes, or macro-

phages revealed that these cells enter inflammatory or damaged brain lesions, particularly around blood vessels¹⁷³. However, monocytes and macrophages do not change into ramified resting microglia¹⁷³. A bone marrow transplantation study with green fluorescent protein (GFP)-expressing cells from GFP-transgenic mice demonstrated the migration and cluster formation of GFP-positive cells and their differentiation and maturation into ramified resting microglia in brain parenchyma¹⁷⁵. These GFP-positive cells clustered in brain parenchyma showed a positive reaction for ER-MP12, a marker of early myeloid cells (GM-CFCs)¹⁷⁵. These data suggest that resting microglia are derived from hematopoietic stem cells via early myeloid progenitors, presumably GM-CFCs.

Results of the above-mentioned studies indicate that resting microglia are derived from proliferating ameboid microglia in the fetal stage and that after birth they are differentiated from microglial progenitors with a proliferative capacity, presumably GM-CFCs, and not from monocytes under normal steady-state conditions (Fig. 3). In the brain under inflammatory or stimulated conditions, activated microglia are found, which are transformed from both ramified resting microglia and reactive (monocyte-derived) microglia.

3) Synovial A cells

Synovial A cells are a macrophage population existing in the synovial membrane of joints. Ontogenetically, they emerge in the late fetal stage¹⁰⁸. Because synovial A cells are absent in *op/op* mice, these cells are classified as an M-CSF-dependent macrophage population; however, their population does not recover after daily administration of M-CSF¹²⁰. Therefore, locally produced M-CSF seems to be essential for the development and differentiation of synovial A cells.

5. Development and differentiation of dendritic cells

1) Heterogeneity and migration of dendritic cells

Dendritic cells are a heterogeneous population and are classified into two major populations: T cell-associated and B cell-associated dendritic cells¹⁷⁶. T cell-associated dendritic cells

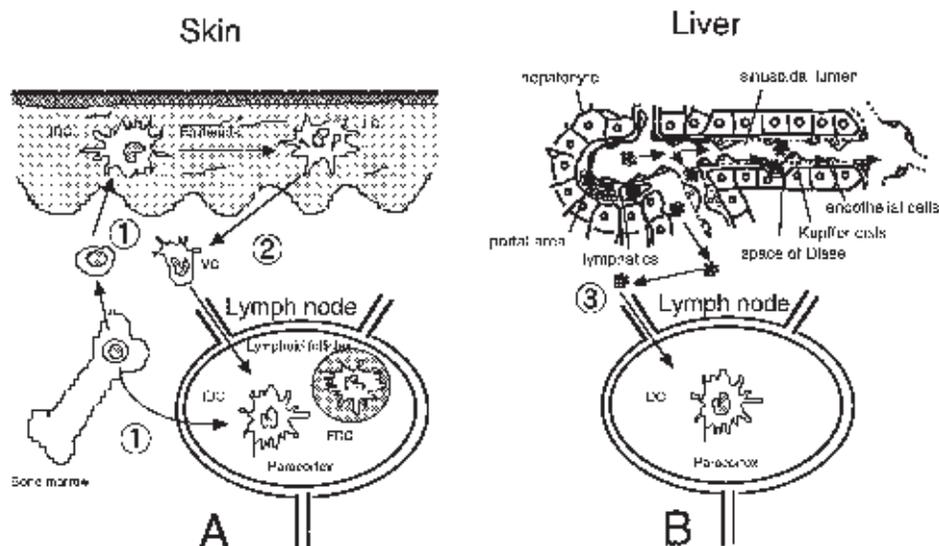


Fig. 6. Development, differentiation, and trafficking of T cell-associated dendritic cells. A: lymphogenous route of dendritic cells from skin to regional lymph node (2), and hematogenous route of dendritic cell progenitors from bone marrow to skin or lymph node (1), B: Blood-lymph translocation of dendritic cells in the liver (3) (LC: Langerhans cell, IDC: interdigitating cell, VC: veiled cell, FDC: follicular dendritic cell).

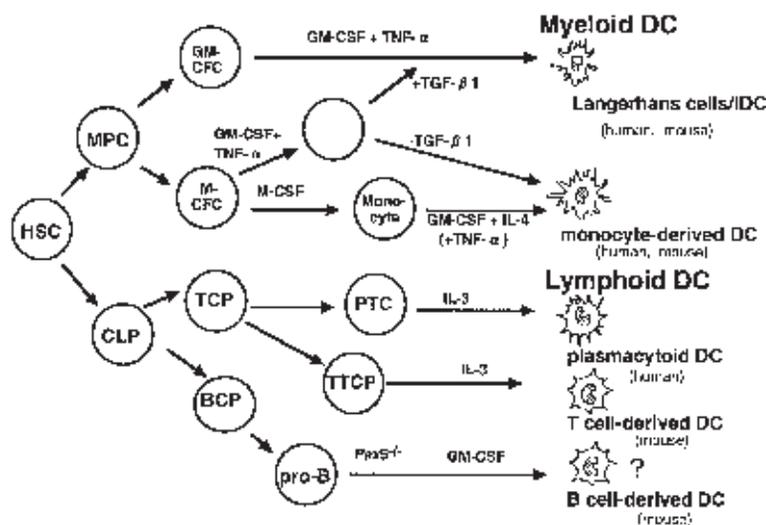


Fig. 7. Development, differentiation, and maturation of dendritic cells (HSC: hematopoietic stem cell, MPC: myeloid progenitor cell, CLP: common lymphoid progenitor, GM-CFC: granulocyte/macrophage colony-forming cell, M-CFC: macrophage colony-forming cell, TCP: T cell progenitor, BCP: B cell progenitor, TTCP: thymic T cell progenitor, PTC: plasmacytoid T cell, IDC: interdigitating cell).

include epidermal Langerhans cells, veiled cells in the dermis (dermal dendritic cells) and afferent lymphatics, interdigitating cells in the paracortical area of lymph nodes or in the T cell-dependent area of peripheral lymphoid tissues, and lymphoid dendritic cells¹⁷⁶. B cell-associated

dendritic cells are follicular dendritic cells (FDCs) located in the germinal center of lymphoid follicles¹⁷⁶. Two theories have been presented for the origin of FDCs: one proposes their derivation from reticulum cells in a follicular germinal center^{10,177}, and the other pro-

poses a bone marrow origin¹⁷⁸⁻¹⁸⁰. However, the former theory has been widely recognized up to the present.

Hence, from the viewpoint of macrophage-related cells, the present author should explain here the maturation and migration of T cell-associated dendritic cells. It is known that epidermal Langerhans cells are differentiated from indeterminate (interdigitating) cells, which are derived via dendritic cell progenitors from hematopoietic stem cells in bone marrow¹⁸¹. During this differentiation process, the epidermal Langerhans cells develop Birbeck granules in their cytoplasm and express E-cadherin. In humans, the monoclonal antibody Lag-1 recognizes mature Birbeck granules¹⁸². Tubulovesicular structures are characteristic of interdigitating cells. The epidermal Langerhans cells migrate into the dermis and become veiled cells (dermal dendritic cells), which then enter the afferent lymphatics and move to the paracortical area of regional lymph nodes (Fig. 6A). Veiled cells extend fan-like processes and are highly mobile. They mature into interdigitating cells *in loco* and lose Birbeck granules from the cytoplasm. Against this view, epidermal Langerhans cells and dermal dendritic cells are currently believed to be different populations¹⁸³. In the lymph nodes, compared with bone marrow-derived dendritic cells, epidermal Langerhans cells and dermal dendritic cells accumulate 3 to 4 times slower, turnover that is dramatically increased by cutaneous inflammation¹⁸³. In normal steady-state conditions, dendritic cells die by apoptosis in the lymph nodes and there are few dendritic cells in the efferent lymphatics and thoracic duct under normal steady-state conditions¹⁸¹. Besides this lymphogenous route from the skin to the regional lymph nodes, there is a hematogenous route for the migration of dendritic precursor cells from bone marrow into lymph nodes and for their differentiation into interdigitating cells in the paracortical area, as demonstrated by the finding of numerous dendritic cells in the lymph nodes at 4 weeks after ligation of the afferent lymphatics draining into the regional lymph nodes in mice¹⁸⁴ (Fig. 6A). Besides both the lymphogenous and hematogenous routes, blood-lymph translocation of dendritic cells occurs in liver¹⁸⁵. In normal steady-state conditions, dendritic cell precursors derived from bone marrow adhere to Kupffer

cells in the hepatic sinusoids, enter the space of Disse, and differentiate into dendritic cells *in situ*¹⁸⁵. These dendritic cells enter the lymphatic vessels of the periportal area of the liver and move into the paracortical area of the hepatic hilar lymph nodes¹⁸⁵ (Fig. 6B).

In adults, although the life cycle of epidermal Langerhans cells is about 16.3 days¹⁸¹, they possess a proliferative capacity and include a long-lived, slowly replicating, self-renewing subpopulation^{186,187}. In contrast, interdigitating dendritic cells in lymph nodes and/or peripheral lymphoid tissues are generally short-lived, existing about 10-14 days in mice and 2-4 weeks in rats, and are constantly supplied by and differentiated from dendritic cell precursors¹⁸¹. Recent studies have revealed two different types of dendritic cell precursors: 1) myeloid dendritic cell precursors and 2) lymphoid dendritic cell precursors. According to the type of precursor cell, the dendritic cells are termed "myeloid dendritic cells" or "lymphoid dendritic cells" (Fig. 7).

2) Multiple pathways of dendritic cell differentiation

Myeloid dendritic cells include Langerhans cells in the epidermis and lymphoid tissues, interdigitating cells in the lymphoid tissues, and veiled cells in the dermis and afferent lymphatics. These myeloid cells are closely related to the dendritic cells developed during ontogeny under unstimulated conditions in the fetus and are derived from myeloid dendritic precursor cells earlier than the stage of monocytes. Monocytes are also differentiated into dendritic cells, which are called "monocyte-derived dendritic cells", in response to inflammatory stimuli.

Myeloid dendritic cells

- (1) Dendritic cells derived from myeloid precursor cells earlier than the stage of monocytic cells

This dendritic cell population includes epidermal Langerhans cells that express CD1a, CD11b, CD11c, CD68, Lag-1, and E-cadherin and are characterized by Birbeck granules in the cytoplasm. In PU. 1-deficient mice, all dendritic cells including epidermal Langerhans cells and interdigitating cells are completely absent, as are macrophages and their related cells and their progenitor cells¹³¹⁻¹³³. Differentiation of all these cells is blocked because of the PU. 1 deficiency. In contrast, in ⁸⁹Sr-induced, severely

monocytopenic, splenectomized mice, the numbers of epidermal Langerhans cells and dendritic cells in thymic medulla and lymph nodes are similar to those of splenectomized control mice¹⁸⁸. Compared with normal littermates, *op/op* mice show no statistically significant difference in the number of dendritic cells in the skin, thymic medulla, lymph nodes, and spleen¹⁸⁹. These results indicate that neither monocytes nor M-CSF contributes to the development and differentiation of dendritic cells in these tissues and suggest that dendritic cells are an M-CSF-independent cell population, presumably derived from GM-CFCs or earlier myeloid precursor cells without passing through the monocytic cell stage^{188,189}. In cultures supplemented with GM-CSF, the presence of proliferating dendritic precursors was demonstrated in peripheral blood and bone marrow, and dendritic cells were generated in *in vitro* studies of murine bone marrow cells with GM-CSF¹⁹⁰⁻¹⁹⁴. In cultures of mononuclear cells from human cord blood supplied with GM-CSF and TNF- α or TGF- β 1, Langerhans cells with Birbeck granules were generated and were demonstrated to be differentiated from CD1a-positive cells originating from CD34-positive hematopoietic stem cells, but not from CD14-positive cells (monocytes)¹⁹³⁻¹⁹⁵. In TGF- α 1-deficient mice, epidermal Langerhans cells are completely absent¹⁹⁵. In *in vitro* studies of CD34-positive hematopoietic stem cells and GM-CSF and TNF- α , Langerhans cells developed and their population grew, particularly when supplemented with TGF- β 1^{195,197}. This type of dendritic cell is called a "myeloid dendritic cell", because, as shown in these *in vitro* studies, these cells are derived from myeloid progenitor cells earlier than the monocytic cell stage, do not pass through the differentiation stage of monocytic cells, and are thus distinct from the cells belonging to the MPS (Fig. 7).

(2) Monocyte-derived dendritic cells

In addition to being derived from myeloid progenitors, dendritic cells are derived from monocytes or macrophages^{192,198-202}. *In vitro* studies have shown that dendritic cells are generated from human peripheral blood monocytes in cultures with GM-CSF and IL-4 and express DR, DQ, CD1a, CD1b, CD1c, CD11, and costimulatory molecules such as intracellular adhesion molecule 1 (ICAM-1), leukocyte function-associated

antigen 1 (LFA-1), B7, or B70; these cells also have T cell-stimulatory activity in autologous and heterologous mixed leukocyte reactions^{192,198}. These dendritic cells are called "dendritic cell-like cells" or "monocyte-derived dendritic cells"¹⁹⁴. In our studies, these dendritic cells showed an ultrastructure of secretory cells resembling epithelioid cells and had neither Birbeck granules nor a tubulovesicular structure¹⁹². However, studies showed that TGF- β 1 induced the development of Birbeck granules in the cytoplasm of monocyte-derived dendritic cells *in vitro* and that these cells expressed E-cadherin and Lag-1, suggesting that monocytes differentiate into Langerhans cells *in vitro*²⁰³. It remains unknown whether this differentiation pathway of monocytes into Langerhans cells occurs as a major route *in vivo* under unstimulated normal steady-state conditions.

In *op/op* mice, the development and differentiation of myeloid dendritic cells are not impaired; however, the development of monocyte-derived dendritic cells is severely impaired in lymph nodes under inflammatory conditions²⁰⁴. In addition, we found that monocyte-derived dendritic cells do not develop in hepatic granulomas induced by Zymosan administration (data not published). In *me^v/me^v* mice, the numbers of epidermal Langerhans cells and interdigitating cells in lymph nodes are reduced with aging, whereas monocytes and monocyte-derived macrophages infiltrate various organs and tissues, accompanied by increased numbers of ER-MP20-positive monocyte-derived dendritic cells in inflammatory foci of lungs, liver, and other tissues¹⁴². Monocyte-derived dendritic cells thus show behaviors distinct from those of myeloid dendritic cells. They develop in inflammatory foci, M-CSF is essential for their development, and their number increases in response to inflammatory stimuli, as shown in studies of *op/op* mice or *me^v/me^v* mice in inflammatory reactions (Fig. 7).

Lymphoid dendritic cells

Lymphoid dendritic cells are a heterogeneous population and at least two subsets have been reported in lymphoid tissues. First, Shortman *et al.* reported in mice that thymic dendritic cells are derived from progenitor cells similar to T cells in the thymus and express major histocompatibility complex class II antigens (Ia

antigens), CD11c, NLDC-145 (DEC205), BP-1, and CD8^{205,206}. The CD8 α -positive, DEC205-positive, CD11b-negative dendritic cells belong to a subtype of T cells and are differentiated from precursor cells weakly positive for CD4, positive for CD44 and *c-kit*, and negative for CD25. IL-3 is important, but GM-CSF is unnecessary for the differentiation of thymic T cell progenitor cells into CD8 α -positive, DEC205-positive, CD11b-negative dendritic cells. The thymic progenitor cells differentiate into T, B, and natural killer cells besides thymic dendritic cells, but not into macrophages. The CD8 α -positive, DEC205-positive, CD11b-negative dendritic cells are distributed from the thymus into lymphoid tissues, particularly the spleen²⁰⁷. CD8 α -negative, DEC205-negative, CD11b-positive dendritic cells are found in the spleen and lymph nodes, which also contain a third subpopulation of dendritic cells: CD8 α -negative, DEC205-positive, CD11b-negative²⁰⁷. In the lymph nodes, compared with the myeloid dendritic cells, the lymphoid dendritic cells have a 30% faster turnover¹⁸³.

In humans, cells previously called plasmacytoid T cells in humans have been thought to be a type of dendritic cell²⁰⁸. These cells resemble plasma cells, express CD4, and are also termed "plasmacytoid monocytes". The plasmacytoid monocytes are IL-3-dependent, differentiate and mature into dendritic cells, and show an intense T cell-stimulatory activity in response to stimuli induced by IL-3 and CD40 ligand. These dendritic cells lack phagocytic activity, do not express any myeloid cell-related antigens such as CD11b, CD11c, CD13, and CD33, and are GM-CSF-independent. Electron microscopically, these dendritic cells resemble plasma cells and extend dendritic cell processes and develop lamellarly arranged rough endoplasmic reticula. The dendritic cells are distributed in the T cell-dependent area of peripheral lymphoid tissues, particularly around high endothelial venules, as well as in the thymus^{208,209}. On the basis of these results, it is speculated that dendritic cell progenitors develop in the thymus, move via the blood stream into peripheral lymphoid tissues, and differentiate into dendritic cells. Some investigators have pointed out the relationship between plasmacytoid T cells and interdigitating cells in lymph nodes and other lymphoid tissues. However, the heterogeneity and lymphogenous route of migration of interdigitating cells must be

considered when discussing the origin of these cells, as mentioned above^{176,181,183}.

In addition, in *Pax5*-deficient mice, pro-B cells are incapable of *in vitro* B cell differentiation, are not restricted to the fate of their lineage, and differentiate into dendritic cells after stimulation with GM-CSF *in vitro*¹⁵⁰. However, this dendritic cell subpopulation has not yet been verified *in vivo*. All these data suggest the presence of differentiation pathways of T or B lymphoid progenitors into dendritic cells, with their differentiation and maturation supported by GM-CSF or IL-3 *in vitro* (Fig. 7). This last-mentioned result should challenge the finding that the development of dendritic cells is not impaired in GM-CSF-deficient mice^{210,211} or in mice deficient in GM-CSF/IL-3/IL-5 β c chain receptor or β IL-3 receptor^{212,213}.

V. Multiple roles of macrophages in inflammation

As mentioned above, there are two major macrophage populations — tissue macrophages and monocyte-derived macrophages — and both populations participate in different stages of inflammation. The liver has a simple structure composed of hepatic parenchymal cells and sinusoidal cells; Kupffer cells are present in the hepatic sinusoidal lumen, and monocytes circulate in peripheral blood. To examine the roles of Kupffer cells and monocyte-derived macrophages population in hepatic granulomatous inflammation, the following various mouse models were have been used. In mice depleted of Kupffer cells by administration of liposome-encapsulated dichloromethylene diphosphonate (lipo-MDP), hepatic granuloma formation is delayed from 3 to 28 days after zymosan injection, accompanied by suppressed expression of M-CSF, IL-1, MCP-1, TNF- α and IFN- γ mRNA, compared with lipo-MDP-untreated control mice²¹⁴. These data imply that Kupffer cells are indispensable for hepatic granuloma formation, play a part act as an initiator, and produce various cytokines including MCP-1 and M-CSF. In mice lacking class A type I and II macrophage scavenger receptors (MSR-A I & II), the development and distribution of Kupffer cells in the liver are similar to those of the wild-type mice²¹⁵. In MSR-A^{-/-} mice, however, hepatic granuloma formation was delayed

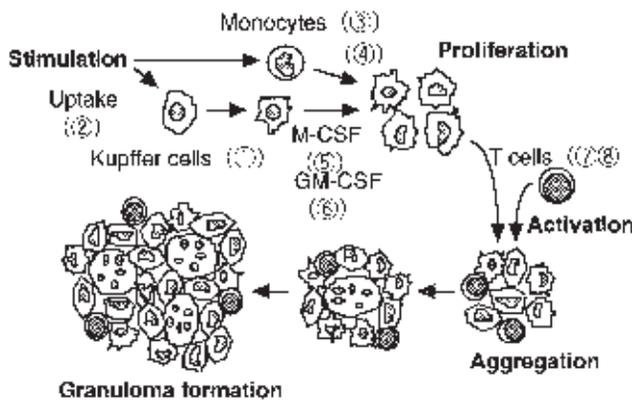


Fig. 8. Schematic representation of macrophage involvement in hepatic granuloma formation in mice. ① Kupffer cell-depleted mice, by administration of lipo-MDP ② MSR-A^{-/-} mice; ③ ⁸⁹Sr-induced monocytopenic mice; ④ CCR2^{-/-} mice; ⑤ *op/op* mice; ⑥ GM-CSF^{-/-} mice; ⑦ nude mice; ⑧ scid mice; ⑨ xid mice.

markedly after intravenous injection of *Corynebacterium parvum* (*C. parvum*), because MSR-A^{-/-} macrophages can not ingest and digest *C. parvum* due to the lack of MSR-A, and their signal transduction through MRS-A for the expression of MCP-1, TNF- α and IFN- γ mRNA is impaired. In the early stage of hepatic granuloma formation, monocyte migration into liver granulomas, phagocytosis of *C. parvum* by and activation of granuloma macrophages are markedly impaired in MSR-A^{-/-} mice.

In ⁸⁹Sr-treated, severely monocytopenic mice, hepatic granuloma formation is severely impaired until a week after Zymosan injection, marked proliferation of Kupffer cells occurs at 5 days, and the Kupffer cells start to collect and aggregate to form granulomas in the hepatic sinusoids and change into epithelioid cells and multinuclear giant cells^{64,216}. Monocytes do not participate in such hepatic granuloma formation; Kupffer cells alone can form hepatic granulomas by their proliferation and differentiation into epithelioid cells and/or multinuclear giant cells. In M-CSF-deficient *op/op* mice, hepatic granuloma formation is delayed early after Zymosan injection, and hepatic granulomas are formed by proliferation of immature Kupffer cells alone, because monocytes are completely or nearly completely absent in peripheral blood and because differentiation of monocytes into macrophages is severely impaired²¹⁷. Although proliferation of Kupffer cells is most important for

hepatic granuloma formation in both monocytopenic mouse models in response to Zymosan stimulation, proliferation of Kupffer cells is more marked in the ⁸⁹Sr-induced, severely monocytopenic mice than in the *op/op* mice.

CC chemokine receptor 2 (CCR2) is a receptor for MCP-1, the most potent chemokine for monocyte chemotaxis. In mice lacking CCR2, monocyte infiltration into Zymosan-induced hepatic granulomas is severely impaired, and hepatic granuloma formation is markedly delayed compared with granuloma formation in control mice²¹⁸. All these data imply that, in addition to Kupffer cells, monocytes are important for hepatic granuloma formation, particularly in its early stage.

GM-CSF is an important growth factor for the proliferation, differentiation, and survival of macrophages, as well as for monocyte chemotaxis. In mice lacking GM-CSF, Zymosan-induced hepatic granuloma formation is delayed in the early stage, and the granulomas disappear more rapidly, compared with wild-type mice. In GM-CSF^{-/-} mice, monocyte influx into liver granulomas and [³H] thymidine uptake rates of granuloma macrophages are impaired in the early stage of hepatic granuloma formation, and the number of apoptotic cells is increased in the granulomas at 8 days after Zymosan injection²¹⁹. GM-CSF^{-/-} mice showed expression of MCP-1, TNF- α , IFN- γ , M-CSF, and IL-1 mRNA in liver granulomas even though there was a total lack of GM-CSF. Thus, GM-CSF plays important roles in monocyte migration into hepatic granulomas and proliferation and survival of granuloma macrophages *in vivo*.

In vitro, T lymphocytes are known to participate in activation of macrophages during the formation of various types of granulomas. To elucidate the roles of T cells in hepatic granuloma formation, we injected Zymosan intravenously into three different types of immunodeficient mice — nude (Hfhllnu/Hfhllnu) mice, C3H/HeN and CB-17/c1r-scid mice, and CBA/N (xid/xid) mice — and compared results with those from control mice^{220,221}. Nude mice are congenitally athymic, have a profound deficiency of T cells, and lack T cell-dependent immune responses in the early stage of adult life. Compared with control mice, in nude mice the number and size of hepatic granulomas and the influx of monocytes into the liver are slightly

reduced and the differentiation, maturation, and activation of granuloma macrophages are delayed. Also, the percentages of Thy 1.2-positive T cells in the granulomas and peripheral blood are lower in the nude mice than in the control mice²²⁰.

The scid mouse is a murine model of T and B cell dysfunction. In these mice, the development and formation of hepatic granulomas are delayed for more than a week after Zymosan injection, the influx of monocytes into granulomas during the early stage of granuloma formation is reduced, and the percentages of Thy 1.2-positive T cells in the granulomas and peripheral blood are lower than in the control mice²²¹. However, [³H] thymidine uptake rates of granuloma macrophages in nude and scid mice do not differ from those of control mice. In both immunodeficient mice, T cell deficiency seems to result in a delay of hepatic granuloma formation because of a failure in the differentiation, maturation, and activation of macrophages *in loco*.

The xid mouse is a murine model of X-linked immunodeficiency, with impaired proliferation and differentiation of B cells. After Zymosan injection, the number of hepatic granulomas increases slightly in xid mice, peaks at 5 days, and rapidly declines from 8 days on²²¹. Also, the influx of monocytic cells and earlier macrophage precursors into granulomas, the proliferative capacity of granuloma macrophages, and the phagocytic and digestive capacity of the macrophages for glucan particles are increased in xid mice compared with control mice²²¹. In xid mice, although the number of T cells in peripheral blood is normal, the percentages of T cells in the granulomas are higher than in the control mice. Such increased percentages of T cells in hepatic granulomas in xid mice seem to result in activation of granuloma macrophages, leading to a rapid disappearance of granulomas via enhanced phagocytosis and digestion of glucan particles by granuloma macrophages. These data for three different types of immunodeficient mice indicate that T lymphocytes are involved in the activation of macrophages in hepatic granuloma formation *in vivo*.

The present author has thus explained the multiple roles of two major macrophage populations, Kupffer cells and monocyte-derived macrophages, in the multistep processes of hepatic granuloma formation, their participation in dif-

ferent stages of granuloma formation, and involvement of various cytokines *in vivo* by the use of various mouse models²²² (Fig. 8).

CONCLUSION

This article has critically reviewed two major theories concerning the development and differentiation of macrophages, the RES and the MPS; pointed out that the development and differentiation of macrophages occurs not in a single cell lineage as proposed by both theories; and explained that macrophages are differentiated through multiple pathways from their progenitor cells via different routes of differentiation. With regard to animal phylogeny, ameboid phagocytic mononuclear cells (macrophages) do develop but monocytes are not detected in invertebrates, whereas both macrophages and monocytes develop in vertebrates. This fact indicates that the development and differentiation of macrophages precede those of monocytes during the evolutionary processes of animals. This phylogenetic principle is applied to human and murine ontogeny of macrophages. In the yolk sac, primitive and fetal macrophages first develop and monocytes are not detected in primitive hematopoiesis, indicating that in early ontogeny these macrophages are differentiated from hematopoietic stem cells, and the developmental stage of monocytic cells is bypassed. In the fetal liver, monocytes develop in permanent, or definitive, hematopoiesis, are released into the peripheral circulation, migrate into fetal tissues, and differentiate into monocyte-derived macrophages in late ontogeny. A subpopulation of primitive and fetal macrophages expresses Ia antigens and differentiates into interdigitating cells in late ontogeny and further into Langerhans cells in perinatal and neonatal life.

In postnatal and adult life, macrophages are differentiated from macrophage precursor cells at different stages or lineages of differentiation originating in bone marrow. In myeloid cell lineages, monocytes or myeloid precursor cells from an earlier stage than the monocytic cells differentiate into macrophages. As advocated by the MPS concept, it has been established that monocytes derived from monoblasts via promonocytes in bone marrow are released into peripheral circulation, migrate into tissues in response to inflammatory stimuli, and differenti-

ate into macrophages *in loco*. This differentiation pathway is supported by M-CSF, is completely blocked in *op/op* mice, and is evident in inflammation. Tissue macrophages are differentiated from macrophage precursor cells earlier than the monocytic cell stage (M-CFCs, monoblasts, promonocytes, monocytes) under normal steady-state conditions. The differentiation pathway of macrophage precursor cells into immature tissue macrophages is supported by growth factors other than M-CSF and is not blocked in *op/op* mice. M-CSF induces proliferation of immature tissue macrophages and their maturation into mature tissue macrophages. Although the differentiation of most tissue macrophages and monocyte-derived macrophages is blocked in PU. 1^{-/-} mice, PU. 1^{-/-} macrophages develop in PU. 1^{-/-} mice that survive when administered antibiotics after birth. Tissue macrophages possess a proliferative capacity, can survive by self-renewal, and are long-lived. Although osteoclasts are an M-CSF-dependent cell population, they are differentiated from preosteoclasts, which are derived from osteoclast progenitors at or before the stage of GM-CFCs, bypassing the stage of monocytic cells. Resting microglia in the hippocampal area of the brain are a M-CSF-independent cell population and are differentiated from microglial progenitors at or before the stage of GM-CFCs, bypassing the stage of monocytic cells. In addition, some macrophage populations are derived from hematopoietic stem cells via B lymphoid progenitors.

Dendritic cells are a heterogeneous population and are differentiated through multiple pathways of differentiation from dendritic cell progenitors: 1) myeloid cells of a stage earlier than monocytic cells, 2) monocytes, and 3) lymphoid progenitors. In normal steady-state conditions, epidermal Langerhans cells and dendritic cells in lymph nodes and lymphoid tissues are differentiated from myeloid precursor cells earlier than monocytes, whereas monocyte-derived dendritic cells appear in inflammatory lesions. Some lymphoid dendritic cells are derived from T lymphoid progenitors or pro-B cells.

Therefore, in addition to the differentiation pathway of monocyte/macrophages as advocated by the MPS concept, there are multiple pathways of differentiation of macrophages and their related cells, some of which bypass the stage of

monocytic cells.

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