NUCLEAR HORMONE RECEPTORS ENABLE MACROPHAGES AND DENDRITIC CELLS TO SENSE THEIR LIPID ENVIRONMENT AND SHAPE THEIR IMMUNE RESPONSE

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Nagy L, Szanto A, Szatmari I, Széles L. Nuclear Hormone Receptors Enable Macrophages and Dendritic Cells to Sense Their Lipid Environment and Shape Their Immune Response. Physiol Rev 92: 739–789, 2012; doi:10.1152/physrev.00004.2011.—A key issue in the immune system is to generate specific cell types, often with opposing activities. The mechanisms of differentiation and subtype specification of immune cells such as macrophages and dendritic cells are critical to understand the regulatory principles and logic of the immune system. In addition to cytokines and pathogens, it is increasingly appreciated that lipid signaling also has a key role in differentiation and subtype specification. In this review we explore how intracellular lipid signaling via a set of transcription factors regulates cellular differentiation, subtype specification, and immune as well as metabolic homeostasis. We introduce macrophages and dendritic cells and then we focus on a group of transcription factors, nuclear receptors, which regulate gene expression upon receiving lipid signals. The receptors we cover are the ones with a recognized physiological function in these cell types and ones which heterodimerize with the retinoid X receptor. These are as follows: the receptor for a metabolite of vitamin A, retinoic acid: retinoic acid receptor (RAR), the vitamin D receptor (VDR), the fatty acid receptor: peroxisome proliferator-activated receptor γ (PPARγ), the oxysterol receptor liver X receptor (LXR), and their obligate heterodimeric partner, the retinoid X receptor (RXR). We discuss how they can get activated and how ligand is generated and eliminated in these cell types. We also explore how activation of a particular target gene contributes to biological functions and how the regulation of individual target genes adds up to the coordination of gene networks. It appears that RXR heterodimeric nuclear receptors provide these cells with a coordinated and interrelated network of transcriptional regulators for interpreting the lipid milieu and the metabolic changes to bring about gene expression changes leading to subtype and functional specification. We also show that these networks are implicated in various immune diseases and are amenable to therapeutic exploitation.

I. INTRODUCTION

The fate of cells is largely determined by the quantitative and qualitative gene expression changes, which are taking place as the result of alterations in endo- and exoge-nous signaling. It is becoming increasingly clear that the major direct initiators and regulators of such changes are transcription factors, which can bring about the changes necessary to alter cellular differentiation and cell fates. Probably the most well-documented cases of cell fate determination processes are examples of cellular differentiation. During these processes, a coordinated set of signals govern the differentiation and cell type specification of stem and/or progenitor cells into diverse directions.

Conceptually, a fairly well understood system is the differentiation of bone marrow-derived myeloid progenitor cells of the immune system such as macrophages or dendritic cells (DCs) (151, 169) amongst others. These cell types are particularly intriguing because they are not just key components of the immune system, but are also pres-
ent in all tissues and many compartments of the body representing a wide array of subtype specification pathways such as classically and alternatively activated macrophages, different tissue macrophages, Kupffer cells, alveolar macrophages, microglia, or among DCs plasmacytoid and conventional DCs, such as Langerhans cells (151, 169). Importantly, these cell types are often exposed to large amounts of lipids, including pathogen- and host-derived lipoproteins or lipids of apoptotic cells; therefore, they represent a well-suited model to study and understand the regulatory principles and logic of the integration of lipid and inflammatory signaling with gene expression at the cellular level (503).

As a general principle, the differentiation and specification of these cells are regulated by endogenous and exogenous stimuli, including cytokines and TLR ligands, respectively (151, 168, 169) (FIGURE 1). However, it is increasingly appreciated that changes in the lipid environment also contribute to cell type differentiation and specification mainly by alterations of transcriptional responses.

In multicellular organisms, a distinct set of transcription factors, the superfamily of nuclear receptors, have evolved to mediate lipid signaling at the level of gene expression (318). Due to the fact that these transcription factors are intimately linked to lipid metabolism, they represent an important crossroad between lipid metabolism and immune function (161, 211, 503). In this review, we focus on a selected group of nuclear hormone receptors, RXR heterodimers (318) (TABLE 1) and evaluate the available evidence on how activation of these receptors can modulate cell fate decisions in macrophages and DCs. How these pathways are interacting with cytokine signaling and how these might be linked to disease states such as atherosclerosis, inflammation, and host response to pathogens will also be discussed.

II. MACROPHAGES ARE IMMUNE CELLS WITH DIVERSE BIOLOGICAL FUNCTIONS

A. Macrophages Are More Than Just “Big Eaters”

It was more than 100 years ago when Elie Metchnikoff discovered macrophages (μαχρος = large and φαγευν = eat) and assigned phagocytosis as their major function.
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Today macrophages represent a very heterogeneous and highly specialized cell population (151). They are involved in many steps of innate and adaptive immune responses, being essential players in antigen presentation, wound healing, elimination of bacteria, removal of necrotic cellular debris, and secretion of cytokines. Moreover, several lines of evidence support a central role for macrophage-mediated inflammation in the pathogenesis of metabolic diseases, most importantly atherosclerosis, but also in diabetes and metabolic syndrome (163, 392).

B. Development of Monocytes/Macrophages

Monocytes constitute ~3–8% of circulating leukocytes and are produced in the bone marrow from hematopoietic stem cell progenitors. After differentiation they are released into the circulation from where they move into tissues usually within 1–3 days. According to a recent report, there is a reservoir for monocytes in the spleen where they form clusters in the cords of the subcapsular red pulp (495).

CD34+ stem cells can give rise to every cell type in the blood. Traditionally, it was believed that monocytes develop from common myeloid progenitors of granulocytes and monocytes termed colony-forming units (CFUs); therefore, the generation of monocytes is part of the general differentiation process of myeloid cells, termed myelopoiesis. Differentiation starts from stem cells with the formation of granulocyte, erythrocyte, monocyte, and megakaryocyte colony forming unit (CFU-GEMM), which give rise to granulocyte, macrophage colony-forming unit (CFU-GM) upon the effect of granulocyte-macrophage colony stimulating factor (GM-CSF). Subsequently, macrophage colony-forming unit (CFU-M or CMP = common myeloid precursor) is induced by macrophage colony-stimulating factor (M-CSF). This develops into monoblast and later promonocyte to finally give rise to monocytes (144, 145). However, the relationship between the origin of monocytes and dendritic cells remains controversial. The existence of a common precursor monocyte DC precursor (MDP) has been shown, but its relationship to common DC precursor (CDP) is still unresolved (see also sect. III B) (139).

There are distinct types of monocytes in the blood: 1) classical monocytes are characterized as CD14++CD16- while 2) nonclassical monocytes show low CD14 and high CD16 cell surface expression (CD14+CD16++) (595). Recent nomenclature distinguishes a third category as well termed intermedier monocytes (CD14++CD16+) (569). CD14+CD16++ monocytes express CC-chemokine receptor 5 (CCR5), whereas CD14++CD16- monocytes express CCR2 (557). This pattern is characteristic for human monocytes. However, murine monocytes are also heterogeneous. CCR2+CX3CR1low cells correspond to the human CD14++CD16- inflammatory monocytes, while resident monocytes are CCR2−CX3CR1hi, corresponding to the human CD14+CD16++ cells (132, 169).

Monocytes can migrate through the endothelium of blood vessels upon various stimuli. During this extravasation they undergo several changes to become macrophages or DCs. Monocytes/macrophages can be characterized by the high level of CD14, CD11b, CD36, CD68 and F4/80 in mice or by epidermal growth factor-like module containing, mucin-like, hormone receptor-like 1, also known as EMR1, lysozyme M, macrophage antigen-1/3, and M-CSF receptor.

The major function of monocytes is related to the immune system: once they differentiate into macrophages and inflammatory DCs, they are either attracted to inflammatory sites or survey the tissues for foreign/accumulated molecules and pathogens to engulf. In response to inflammatory stimuli, monocytes are rapidly recruited to the sites of inflammation where they are involved not only in the killing/elimination of such particles but evoke specific signaling processes to activate further immune mechanisms. Macrophages are also capable of activating adaptive immune responses, whilst DCs are considered to be more efficient in this. Another important role for macrophages stems from their potency on metabolizing lipid molecules. Probably as part of its original protective role against foreign substances, macrophages are also capable of recognizing lipid molecules when accumulating within the tissues. This process linked macrophages to metabolism, and roles have been assigned for them in the process of atherosclerosis and lipid storage diseases. Recently, by studying the crosstalk of immune system and lipid metabolism, macrophages have been involved in the process of obesity and insulin resistance as particular cell types regulating lipid metabolism and inflammation in adipose tissue (392; for details, see below).

The differentiation of myeloid cells is principally regulated by cytokines and orchestrated via cooperative gene regulation by transcription factors. The most important players are PU.1, CCAAT/enhancer binding proteins (C/EBPs), and AP-1. For a detailed overview of transcriptional control of granulocyte and monocyte development, we refer to other reviews (144, 145). Briefly, at early stages of hematopoiesis, PU.1 directs stem cells to the lymphoid-myeloid lineage by inhibiting megakaryocyte-erythroid differentiation via interacting with GATA-binding protein (227, 330, 465). In the next step C/EBPα inhibits lymphoid, whilst induces granulocyte-monocyte development (589, 590). PU.1 again induces monocytic commitment (107, 278). PU.1 itself is induced by C/EBPα (550) and activated by c-Jun (37). C/EBPα together with c-Jun or c-Fos favors monocytic development (294, 308, 496), which is further assisted by protein kinase C (PKC), Egr-1, Egr-2, VDR, MafB/c, and
interferon regulatory factor 8, while RAR and C/EBPβ direct granulopoiesis. Phorbol esters are widely used as inducers of monocytic maturation of myeloid cell lines. They activate PKC-α, which in turn activates c-Jun kinase 2, that will activate c-Jun and induce monocytic maturation (413).

C. Macrophage Activation and Function in Innate Immunity

Macrophages are central players of innate immunity not only because they eliminate pathogens, cellular debris, and senescent and dying cells, but they also produce a range of secretory products, which affect the migration and activity of other immune cells. Macrophages are inherently heterogeneous, as they need to respond to a variety of signals in very different tissue contexts. One level of heterogeneity originates from the localization of the resident macrophages. There are specialized macrophages with more or less different functions, like Kupffer cells in the liver, microglia in the central nervous system, osteoclasts in the bone, alveolar macrophages in the lung, perivascular macrophages, and Hofbauer cells in the placenta, etc. (168, 169) (FIGURE 2). Another level of heterogeneity is initiated by environmental signals, most importantly cytokines and pathogen-derived molecules. Initially, activated macrophages were defined as cytokine-producing cells that are able to kill pathogens (108). However, the changing inflammatory milieu could result in distinct activation of cells leading to subpopulations with specialized functions. The distinct functions require highly specialized cells, which are differentiated upon cytokines and microbial products (312). Pro-inflammatory molecules like interferon-gamma (IFN-γ), tumor necrosis factor (TNF), and activators of pattern recognition molecules like Toll-like receptors (TLRs) induce the so-called classical activation of macrophages (classically activated macrophages, or M1 on FIGURE 4C). Consequently, macrophages migrate to the sites of inflammation to engulf and degrade pathogens by producing nitrogen-monoxide and radicals. They also secrete proinflammatory molecules such as TNF, interleukin (IL)-1, IL-6, and MCP-1 to sustain the inflammatory reaction. This classical pathway of IFN-γ-dependent macrophages activation provokes Th1-type responses and serves as an efficient arm of innate immunity directed against intracellular pathogens, like Listeria monocytogenes or Mycobacterium tuberculosis (165, 353).

As a result of their studies on mannose receptor, Gordon and colleagues (484) discovered another class of activated macrophages, which they termed alternatively activated macrophages (or M2 on FIGURE 4C). Alternative macrophages, in contrast to the classically activated cells, express high levels of mannose receptor and exert an almost opposite immunophenotype. They cannot produce nitrogen-monoxide and radicals required for killing, they inhibit T cell proliferation (459), and they provoke immunotolerance or Th2 immune responses (104). They can also produce anti-inflammatory molecules such as transforming growth factor (TGF)-β, IL-10, or IL-1 receptor antagonist (138, 165, 459) and inhibit the secretion of pro-inflammatory molecules such as IL-1, TNF, IL-6, IL-12, and macrophage inhibitory protein (MIP)-1α (50, 86, 482). Alternatively activated macrophages can be characterized by high levels of mannose receptor (484), CD23 (34), alternative macrophage activation-associated chemokine 1 (AMAC-1 or CCL18) (264), arginase-1 (361), FIZZ1, and YM1, (421). The alternative activation of macrophages is induced by Th2 cytokines IL-4 and IL-13 and results in a distinct macrophage subpopulation with roles in humoral immunity and resolution such as repair, wound healing, angiogenesis, tissue repair, and extracellular matrix deposition (164, 168, 173, 264, 265, 353). Under in vivo conditions, alternatively activated macrophages are found at border surfaces in the body such as lung, placenta, and perivascular space (75, 136, 264, 300, 358).

However, a growing body of evidence suggests that the group of nonclassically activated macrophages itself is heterogeneous and exhibits significant differences in their biochemistry and physiology. Therefore, some authors suggest that, based on their functions, macrophages should be classified into three major groups: classically activated macrophages (playing important roles in host defence), wound healing macrophages, and regulatory macrophages. In addition to these categories, there are macrophages that share characteristics of two groups, e.g., tumor-associated macrophages, which have many characteristics of regulatory macrophages but also share some characteristics of wound-healing macrophages (353, 354).

D. Antigen Presentation by Macrophages: Induction of Acquired Immunity

Macrophages phagocytose self-proteins and cells during normal tissue repair and aging. These self-proteins do not activate T cells, because in the absence of infection, macrophages in general express low levels of major histocompatibility complex (MHC)-II and costimulatory molecules. However, lipopolysaccharide (LPS) and various cytokines stimulate macrophages to upregulate MHC-II and B7 (CD80 and CD86 together) molecules, providing these cells with strong antigen presentation properties. This is complemented by their ability to uptake and process antigens. In addition, macrophage activation results in upregulation of cytokines such as IL-1, IL-6, IL-8, IL-12, and TNF (324, 529). With these capacities, macrophages are able to activate memory and effector T cells, but not naive T cells. Interestingly, a subset of specialized macrophages (subcapsular sinus macrophages) contribute to early delivery of antigens to B cell follicles at the subcapsular sinus of...
lymph nodes (69, 411) by collecting and distributing native antigens. It should be mentioned that macrophages, in particular, alveolar macrophages and tumor-associated macrophages, have been linked to suppression of T cells as well (363, 577). Clearly, macrophage subtype specification has an important role in determining the direction of the immune response, and thus macrophages are important amplifiers of the adaptive immune response.

E. Macrophages in Metabolic Processes

There is a rich array of interactions between inflammation and metabolism. Pathogens and inflammation itself could disturb metabolic processes, and vice versa, chronic metabolic diseases could lead to abnormal immune reactions. In some cases, it is hard to deconvolute what is cause and what is consequence. Metabolic syndrome, a constellation of metabolic abnormalities including insulin resistance, hypertension, dyslipidemia, and central obesity, is very often coupled to atherosclerosis, a progressive, degenerative disease of blood vessels. Atherosclerosis is a good example of the intersection of metabolic and immune processes, especially if one considers the pivotal role of macrophages. The progression of the atherosclerotic lesion starts after birth, and generally, the first clinical manifestations occur in the sixth decade of life. Complications of atherosclerosis such as ischemic heart disease, myocardial infarction, and stroke make atherosclerosis responsible for most of the deaths in western societies. The first sign of lesion formation in the vessel wall is the lipid/cholesterol accumulation observed by the appearance of fatty streaks within the subintimal tissues (175) (FIGURE 6). Cholesterol accumulation is driven by pathogenic interaction between circulating lipoproteins, hemodynamic factors, endothelial cells, vascular smooth muscle cells, macrophages, and T lymphocytes and results from the abnormal handling of lipids by macrophages (163, 311, 475).

Vessels are covered by a single cell layer, the endothelium, that serves as a barrier between the circulating blood and subendothelial tissues. Molecules and cells have to migrate through this layer to reach their destination in a regulated fashion. Preferred sites where atherosclerotic lesion starts to develop are places with vulnerable endothelium, i.e., where the cell layer is injured or gets injured easier than at other sites. A disturbance in the laminar flow of blood is, for example, one such factor that increases endothelial vulnerability. Independently of the cause, endothelial dysfunction results in the accumulation of low-density lipoprotein (LDL) in the subendothelial matrix (311). It is not clear how LDL gets modified, but this leads to the appearance of minimally oxidized/modified LDL (mmLDL) and subsequently fully oxidized LDL (oxLDL) containing multiple oxidized lipid molecules (163). Oxidatively modified lipoproteins and lipids can activate both endothelial cells and monocytes/macrophages leading to the migration of monocytes into the subendothelial space (420). Here, macrophages start taking up and clearing up the accumulating lipid particles. However, this might be incomplete and/or the continuous supply from the circulation overloads the lipid handling capacity of the macrophages leading to chronic inflammation with further accumulation of lipids and involvement of multiple cell types, such as endothelial cells, additional macrophages, granulocytes, lymphocytes, fibroblasts, and smooth muscle cells from the media of the vessel. The release of inflammatory mediators and chemotaxants by these cells evokes a characteristic inflammatory response around lipid accumulation by attracting additional cells to the lesion (377, 420, 485).

Macrophages are considered to be the first cellular components in lesion formation. They take up lipid particles through specialized cell surface proteins termed scavenger receptors (SRs), such as SR-A and CD36. Importantly, these scavenger receptors are not subjected to downregulation via a negative-feedback mechanism such as in the case of LDL receptor. Consequently, the more lipid molecules that are present, the higher the lipid concentration in the cells will be leading to the appearance of the characteristic macrophage subtype, the lipid-loaded foam cells. These cells can be identified already in the fatty streaks. Macrophages not only take up lipid particles from the environment, but they metabolize them and eliminate lipids through their ATP-binding cassette transporters such as ABCA1 and ABCG1 towards high-density lipoprotein (HDL). If there is an imbalance between the amount of lipids taken up and the metabolizing/effluxing capacity of the macrophages, they start accumulating lipids resulting in increased cell death and sustained chronic inflammation (163, 190, 298). As a consequence of the chronic inflammation, smooth muscle cells migrate from the tunica media and go through a fibroblast-like transformation resulting in proliferation and production of extracellular matrix elements. This leads to the formation of the late, fibrous atherosclerotic plaques. If calcification (sclerosis) occurs, the artery wall becomes rigid and fragile. Finally, the originally stable lesion may change

FIGURE 2. Development and in vitro models of macrophages and dendritic cells (DC). A: development and the most important subtypes of macrophages and DC. Macrophages and DC can be found in many tissues, and they can play different functions. Conventional DC, plasmacytoid DC, and monocytes share a common progenitor, the macrophage and DC precursor (MDP). However, recent data indicate that some macrophage and DC subtypes, e.g., microglial cells and Langerhans cells, are not necessarily derived from this common bone marrow progenitor. B: in the two most common in vitro models, macrophages and DC are differentiated from murine bone marrow cells and human monocytes in the presence of growth factors and cytokines. Activated macrophages and mature DCs usually are obtained by using Toll-like receptor (TLR) ligands and/or various cytokines.
into an unstable vulnerable plaque that can easily rupture the endothelium leading to the formation of thrombus and intravascular coagulation.

Nuclear receptors, most importantly PPARγ and LXRα, have been implicated in the regulation of lipid metabolism in several cell types contributing to lesion initiation, development, and progression. These include macrophages, fibroblasts, T lymphocytes, smooth muscle cells, and endothelial cells. We will detail the role and contribution of PPARγ and LXR in the formation of atherosclerotic lesions (41, 73, 161, 211, 501, 523) in sections VI and VII.

Another metabolic condition in which the involvement of macrophages has been highlighted in recent years is metabolic syndrome itself (for a review, see Ref. 392). It is an emerging concept that inflammation appears to be a component of obesity-associated insulin resistance. Several lines of evidence including genetic as well as pharmacological inhibition of pathways contributing to inflammatory responses were found to protect against diet-induced insulin resistance. In addition, it has been shown that chronic activation of intracellular pro-inflammatory pathways in target tissues of insulin leads to obesity-related insulin resistance. Another relevant feature of the disease condition is that macrophages are present in adipose tissue of obese individuals more than in lean ones. Moreover, these cells appear to be the major sources of inflammatory cytokines linked to insulin resistance such as TNF-α, IL-1β, IL-6. This leads to the activation of Jun NH₂-terminal kinase (JNK) (213, 460). Since it has been suggested that adipose tissue from obese mice and humans are infiltrated with increasing number of macrophages (560, 571), it represents a major new paradigm potentially explaining the disease process.

According to some reports, more than 40% of the total adipose tissue cell content from obese rodents and humans can be macrophages, while in lean counterparts this value is ~10% (559). The suggestion is that adipose tissue macrophages or ATMs are the major sources of cytokines, which can function as paracrine and/or endocrine mediators of inflammation leading to decreased insulin sensitivity. It is believed that activation of macrophages leads to the production and release of chemokines and cytokines such as IL-1β, TNF-α and IL-6. This leads to the activation of Jun NH₂-terminal kinase (JNK) (29, 202), inhibitor of κB kinase β (IKKβ), and other kinases (224). The insulin-resistant state is characterized by increased JNK and IKKβ levels in adipose tissue and skeletal muscle. This in turn leads to activation of AP-1 and NF-κB and the subsequent transcriptional activation of inflammatory genes. Insulin receptor substrate also gets phosphorylated, leading to cellular insulin resistance. Nuclear receptors, most prominently PPARγ, have been linked to these macrophages and to disease progression as it will be discussed in section VII.

### III. The Main Functions for Dendritic Cells Are Antigen Presentation and Lymphocyte Activation

#### A. Antigen Presentation by Dendritic Cell Is a Key Step in Engaging the Adaptive Immune Response

Traditionally, macrophages and B cells were thought to be the only cell types able to present antigens to T cells and thus elicit immune response. However, Steinman and Cohn (487) showed that another kind of antigen-presenting cell types, DCs, are indispensable for the initiation of the adaptive immune response (28). DCs can be considered as effector cells with the potential to interact with lymphocytes and regulate their function (430). DCs also play an important role in innate immune responses, e.g., plasmacytoid DCs produce type I IFNs in response to viral infection, while TNF-α/inducible nitric oxide synthase (iNOS)-producing DCs mediate defense against bacterial infection (466, 486). Moreover, in addition to eliciting immune response, DCs could also provoke immunological tolerance by inducing deletion or anergy; thus DCs contribute to limiting autoimmunity (96).

DCs can be found in lymphoid organs, in the blood, at body surfaces (e.g., the skin, pharynx, upper esophagus, vagina, and anus), and at mucosal surfaces such as the respiratory and gastrointestinal system (486) (Figure 2). Importantly, DCs can be generated in vitro from precursors found in the bone marrow, spleen, or blood (monocyte-derived DC), allowing studies using large amount of cells (222, 452, 568) (Figure 2). Similarly to macrophages, DCs form a heterogeneous cell population, and DC subsets differ in function, location, migratory pathways, and activation status. Remarkably, we do not have any good phenotypic markers specific to DCs, nor any functional criteria; therefore, the clear distinction of DCs from macrophages is still problematic (151, 220).

Early studies, using mainly Langerhans cells (DCs in the epidermis), demonstrated that DCs exist in two states: “off” (immature) and “on” (mature) (430). The paradigmatic Langerhans cell life cycle and migratory pathways were modeled based on studies carried out in the 1980s (246, 430, 540, 567). According to this model, migratory DCs such as Langerhans cells, work at the interface of peripheral tissues. These cells are sentinels of the immune system, and they sense and translate environmental cues by sampling and processing extracellular and intracellular antigens. DCs uptake antigen by various mechanisms (383, 456) and migrate to lymph nodes, where they present antigens and stimulate T cells. The pathogen-derived signals, pro-inflammatory cytokines, and danger signals all integrated by DCs and define the immune response. These ac-
tivating stimuli lead to the maturation of DCs and promote the transition from an “antigen-sampling mode” to the “antigen-presenting mode” (430). Mature DCs deliver three types of signals that are thought to be essential to initiate T-cell activation and determine the fate of naïve T cells: antigen presentation (signal 1), costimulation (signal 2), and polarizing cytokines (signal 3) (247, 430).

The “Langerhans cell paradigm” has been a guiding principle in DC research (246, 430, 540, 567). However, it is clear now that this model does not describe the function and life cycle of several DC subsets. Three examples are given here to illustrate the functional heterogeneity of DCs and the differences from the original paradigm.

First, recent studies suggest that Langerhans cells are dispensable for T cell priming, and in vivo mouse Langerhans cells appear to suppress the immune responses (151, 246, 259). These data are paradoxical considering that Langerhans cells are featured in many textbooks as prototypical DCs.

Second, DCs in secondary lymphoid tissues are not necessarily mature and not derived from cells previously resident in peripheral tissues. Instead, many DCs in the spleen and lymph nodes, especially in the steady state, are immature and derived from blood-born progenitors (430, 540, 541, 567).

Third, DCs that express maturation markers are not always immunogenic (the maturation concept is reviewed in details in Ref. 430). Tolerogenic DCs are known to express substantial level of costimulatory molecules, and other maturation markers and certain environmental signals seem to mature DCs into a tolerogenic mode.

B. Development and Origin of Dendritic Cells

Several DC subsets have been identified in both mice and humans. These subsets are distinguished by marker expression, function, location in the body, and whether they are generated in the steady state or during inflammation. Two classes of DCs are present in the steady-state plasmacytoid DCs and conventional DCs (FIGURE 2). Conventional DCs can be further subclassified into migratory and lymphoid-resident DCs. The precursor-progeny relationship of monocytes, macrophage, and DCs has been debated ever since the discovery of DCs. There is also a significant difference between mouse and human DC subtypes, which must be taken into consideration. Recent data indicated that both the plasmacytoid and the conventional lymphoid resident DCs derived from a common bone marrow progenitor. A CDP (common DC precursor) was identified from bone marrow that efficiently generated in vitro and in vivo plasmacytoid and conventional DCs but not other cell lineages (372, 393). However, other researchers described that conventional DCs, monocytes (139), and even plasmacytoid DCs (24) share a common progenitor, the MDP (macrophage and DC precursor), suggesting that these cells have a common origin. The ontogenetic relation between MDP and CDP is controversial. These progenitors probably represent different stages of differentiation along the same pathway. Indeed, a recent report suggested that MDPs produced monocytes and CDPs, and CDPs are further restricted to generate conventional DC precursors (pre-cDCs) and plasmacytoid DCs (301). Plasmacytoid DCs are profoundly different from conventional DCs. These cells live longer and harbor characteristic immunoglobulin rearrangements. They can be found in bone marrow, in the blood, and on the periphery and are specialized to respond to viral infection by producing massive amounts of IFN-γ. Plasmacytoid DCs are also involved in antigen presentation and induction of tolerance.

Moreover, it was demonstrated that at least a few subsets of tissue resident DCs (inflammatory DCs) derived from monocytes in vivo (152, 423). In addition human blood monocytes are a commonly used precursors for ex vivo generation of DC (monocyte-derived DC) (452).

Consistent with the common origin of macrophages and DCs, several well-established transcription factors, which were considered as a macrophage specific regulator, are also important for DC development. For example, PU.1 transcription factor is required for myeloid DC development (18, 179). Together these results suggested that probably a similar set of transcription factors and signaling pathways shapes the differentiation and function of DCs and macrophages.

The molecular details of the regulation of DC differentiation are still poorly characterized, although it was described that several cytokines and transcription factors are necessary for DC development (570, 588). Flt3 ligand is critical for the development of both conventional and plasmacytoid DC differentiation; in contrast, GM-CSF promotes only conventional DC development (570). In addition, in humans, treatment with IL-4 and GM-CSF cytokine is used for the ex vivo generation of monocyte-derived DCs (452). Much less is known about the transcriptional regulation of DC development, and only a few putative “master” transcription factors have been identified so far (588).

Differentiation, lineage development, and also subtype specification is known to be controlled by various cytokines in the case of both macrophages and DCs. However, this process is very much influenced by the extra- and intracellular lipid milieu (FIGURE 1). In the rest of this review, we will summarize what is known about the contribution of lipid-activated transcription factors, nuclear hormone receptors to these processes. Due to the large amount of data
IV. RXR AND ITS PARTNERS: RAR, LXR, PPAR, AND VDR

The nuclear receptor superfamily includes both steroid and nonsteroid receptors (318). Most of these proteins bind small lipophilic molecules, which either enter the cells or are generated inside the cell (FIGURE 3). One-third of the 48 human nuclear receptors form heterodimers with RXR (78, 154). Among these RXR partners, there are “classical” endocrine receptors, e.g., thyroid hormone receptor (TR), RAR, and VDR, which are activated by high-affinity ligands, such as thyroid hormones, all-trans-retinoic acid, and 1α,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], respectively. Another group of RXR partners consists of “adopted orphan” receptors, e.g., LXR, PPAR, and farnesoid X receptor (FXR). These receptors are activated mainly by metabolites, like oxysterols, fatty acids, and bile acids, which bind their receptors with low affinity (318, 473). Most RXR partners require RXR as an obligatory dimerization partner for DNA binding and transcriptional activity (FIGURE 3). As a general principle, RXR heterodimers are believed to reside in the nucleus bound to directly repeated response elements with half site sequence AGGTCA or a variant of it. RXR heterodimers are also believed to repress transcription in the absence of a ligand by recruiting a repressor complex. Upon ligand binding, transcriptional activation takes place as a result of a molecular switch replacing the corepressor complex with coactivator complex(es) (368). In addition, ligand-bound receptors can contribute to gene expression regulation without binding DNA by a phenomenon termed transrepression. In such cases, the receptor interferes with the activity of other transcription factors by protein-protein interactions combined with posttranslational modifications. Most prominently some of the anti-inflammatory activities of various RXR heterodimers are believed to use such pathways (FIGURE 3).

Although many other nuclear receptors [e.g., glucocorticoid receptor (GR) and estrogen receptor (ER)] are also expressed and have documented immunomodulatory role in macrophages and DCs, in this review we will focus on RXR and its partners: RAR, LXR, PPARγ, and VDR (TABLE 1). We will not cover the potential role of other RXR partners, e.g., Nurrl, PXR, CAR, and PPARδ/β, because they are not expressed in these cells or have not been investigated in sufficient detail. In this section we cover the general features (e.g., receptor isotypes, ligands, knockout phenotypes, and general functions) of RXR and its partners, RAR, LXR, PPAR and VDR, while in subsequent sections we review the specific roles for these receptors in macrophages and DCs. Finally, we will discuss the interactions between the various pathways and also how regulated ligand production contributes to lipid signaling in these cell types.

A. Retinoic Acid Receptors (RARs and RXRs) Are Activated by Vitamin A Derivatives

Vitamin A and its derivatives, retinoids, have profound effects in development, differentiation, homeostasis, and various aspects of metabolism. The discovery of retinoid receptors substantially contributed to the understanding of how these small, lipophilic molecules, most importantly retinoic acid (RA), work as a morphogenic hormone and exert their pleiotropic effects (159, 307, 336, 410). There are two families of retinoid receptors, RARs and RXRs, that are capable of recognizing retinoids specifically and exert their biological effects by regulating gene expression (112). Both families contain three isotypes: α, β, and γ encoded by separate genes and giving rise to numerous alternatively spliced variants (60, 74, 249, 306, 317–319, 584).

RARs can be activated by all-trans-retinoic acid (ATRA) and 9-cis-retinoic acid (9-cis RA), while RXRs can be activated only by 9-cis RA (200, 292). There are natural and synthetic compounds with at least some selectivity for RXR (33, 49, 283, 292) termed retinoids (359).

RARs have been implicated in embryonic (336), skeletal, (307), and myeloid development (248, 250); in wound healing; in keratinization (172); and also in the developing nervous system (491). RARα, RARβ, and RARγ null mice are viable. They display some aspects of the fetal (and postnatal) vitamin A deficiency (VAD) syndromes (566). For a detailed overview, we refer the reader to a recent review (323).

RXR has a unique role among nuclear receptors because it forms heterodimers with other nuclear receptors (60, 287). Therefore, ligand activation has potentially pleiotropic effects on numerous biological pathways and has been implicated not only in retinoid responses but also in various metabolic pathways. RXR was originally identified as a novel retinoid-responsive transcription factor (319). However, it turned out that RAR was more similar to the TR than to RXR, suggesting that these two retinoid receptors are implicated in distinct pathways instead of representing a redundant retinoid signaling pathways. The lack of RXRα results in embryonic lethality in homozygous embryos (249, 490). The abnormalities observed in RXR null mice were highly similar to the effects of embryonic VAD (251).

One of the most enigmatic areas of the nuclear receptor field is the existence of endogenous RXR activators and...
FIGURE 3. The principles of ligand generation and nuclear receptor action. A: biologically active ligands of nuclear receptors can be derived from the surrounding environment, from the bloodstream, or can be generated endogenously from their precursors. B: prototypic natural ligands of RXR and its partners are shown. C: active ligand or its precursor enters the cell and gets into the nucleus where it binds the receptor. Precursors go through enzymatic transformation(s). Liganded receptors can recruit coactivator and corepressor complexes to activate or repress transcription, respectively. For gene regulation, RXR heterodimers bind specific DNA sequences, called hormone response elements. Alternatively, nuclear receptors might interfere with the activity of other transcriptional factors, such as NF-κB and AP-1.
RXR-specific signaling. Several molecules have been identified as potential endogenous ligands for RXR: 9-cis RA (200), phytanic acid (290), and docosahexaenoic acid (116), but none of these has been proven to be the bona fide endogenous activator so far. Chambon and colleagues (66) excluded 9-cis RA as the RXR ligand during keratinocyte differentiation by using a combination of RAR and RXR conditional knockout mice. Perlmann and colleagues (478) created a ligand detector mouse line. They constructed transgenic lines using Gal-DNA binding domain and RXR-ligand binding domain fusion protein, a reporter gene, β-galactosidase containing Gal binding sites in the promoter. X-gal staining of mouse embryos revealed sites of ligand production. Staining of specific regions in the spinal cord suggested the production of endogenous ligand. Luria et al. (310) used a similar system with green fluorescent protein as a reporter (310). They also obtained reporter/RXR activity in spinal cord and upon exogenous ligand treatment in the brain and in the olfactory epithelium.

Due to the fact that RXR works as a general dimerization partner for many nuclear receptors (60, 261, 271, 287), the other intriguing question concerning RXR is if there is a heterodimer-independent RXR signaling. There are in vitro data showing the presence of RXR homodimers and tetramers, but under in vivo circumstances, no RXR homodimer has been detected (84, 257, 542). This is probably due to the strong binding capacity of RXR monomer to its dimerization partners, and until such free partner is present, no RXR-RXR interaction occurs. Our recent gene expression profiling provided some clues though regarding heterodimer-independent RXR signaling in DCs (508). Importantly, RXR appears to be an ancestral nuclear receptor from which many of the other receptor families emerged. Ligand-binding domains of the RXRs from different species show high degree of similarity from jellyfish to human, and all are capable of binding 9-cis RA (268). The more advanced arthropods including the insects have no RXR per se but express ultraspiracle (USP), a homolog of the RXR (195, 396). These suggest that RXR signaling appeared before RAR, PPARs, and LXRs emerged, suggesting the persistence of autonomous RXR signaling. However, it is also possible that evolution generated multiple partners for the RXR, which can act only through its partners today.

**B. LXRs Are Cholesterol Sensors**

LXRs are involved in many cholesterol- and bile acid-related biological and pathological processes. LXRα and -β were cloned in 1994 based on sequence homology with other nuclear receptors from a liver-derived cDNA library. LXRα is highly expressed in the liver and at lower levels in adipose tissue, intestine, lung, kidney, the adrenal glands, and macrophages. LXRβ is ubiquitously expressed (479, 565). Three LXRα isoforms have been reported so far, all derived from the same gene via alternative splicing and differential promoter usage (80).

As far as the regulation of the receptors are concerned, in human but not in mouse cells LXR can induce its own transcription (275, 295, 562). With the study of the promoter of LXRα, CCAAT/enhancer-binding proteins (C/EBPs) were reported to isotype and cell-type specifically regulate LXRα expression (483). Remarkably, PPARγ can also induce the expression of LXRα (77). Interestingly, resveratrol, a naturally occurring polyphenol, was also shown to regulate the expression of LXRα in human macrophages, which could be a possible molecular explanation for some beneficial effects of polyphenols in cardiovascular diseases (468).

LXRs, similarly to PPARs, have large hydrophobic cavities that enable the binding of several different ligands with lower affinity (58, 382, 530). A number of oxysterols have been identified as potential endogenous ligands for LXR. 24(S)-hydroxycholesterol, 25-hydroxycholesterol, 22(R)-hydroxycholesterol, and 24(S),25-epoxycholesterol were shown to bind to and activate LXRs at physiological concentrations (231, 284, 404). Several other cholesterol metabolites have been reported recently as potential LXR activators. For a detailed overview of LXR activator oxysterol metabolites, we refer to a recent review (523). The search for endogenous LXR activators placed oxysterol-producing enzymes in the focus of LXR research. One of the candidates, CYP27, is a p450 enzyme that generates 27-hydroxycholesterol, which can activate LXR (148, 230, 231, 498). CYP27 is induced during macrophage development and is a direct target for RAR, RXR, and PPARγ receptors (498).

Recently, Mitro et al. (339) assigned an unexpected role for LXR, by showing that the receptor could function as a potential glucose sensor and glucose could activate LXR-dependent gene expression. However, others reported that glucose is required for the transcription factor carbohydrate-responsive element-binding protein and that LXRs are not necessary for the induction of glucose-regulated genes in liver (120). Therefore, the contribution of glucose to LXR activity requires further studies.

There are two widely used synthetic LXR agonists. T0901317 is a nonsteroid LXR activator (435), which also activates farnesoid X receptor (FXR) and pregnane X receptor (PXR) (214, 340). GW3965 is another potent nonsteroid LXR-selective activator (92).

These molecules usually do not discriminate between LXRα and LXRβ. However, LXRβ-selective agonists also exist. N-acylthiadiazolines show increased selectivity for LXRβ (345). Despite the selectivity and modest potency, the com-
The major function for LXR is the regulation of lipid metabolism at various levels. Activation of the receptor induces the expression of sterol regulatory element binding protein 1c (SREBP-1c), a central regulator of fatty acid synthesis (117, 434, 462, 579). In addition to SREBP-1c, LXRs regulate several other enzymes involved in lipogenesis such as fatty acid synthase (239), acyl-CoA carboxylase (512), and stearoyl-CoA desaturase 1 (551). When mice were treated with LXR agonists, increased hepatic and plasma triglycerides were observed, generating a serious limitation on the therapeutic introduction of LXR agonists (88, 174, 462).

LXRs do not only regulate the synthesis of lipid molecules, but their target genes are involved in the regulation of lipid transport as well: lipoprotein lipase (LPL) (591), cholesteryl ester transfer protein (309), phospholipid transfer protein (PLTP) (67, 274, 313), apolipoprotein E (276), and the apolipoprotein C-I/C-IV/C-II gene cluster (314).

Furthermore, LXR induced genes are involved in the uptake of lipoprotein particles, like low-density lipoprotein receptor (LDLR) (223) and scavenger receptor BI (SR-BI) (315, 582).

LXRs also control the expression of members of the ATP-binding cassette (ABC) superfamily of membrane transporters such as ABCG5 and ABCG8 (433, 536) in the intestine or ABCA1 and ABCG1 in macrophages (99, 255, 435, 450, 464).

In vivo consequences of LXR activation have also been studied. Administration of a synthetic LXR agonist T0901317 induces expression of LXR target genes, like ABC transporters and lipogenic genes, and results in increased triglyceride and phospholipid levels and in hepatic steatosis in mice (88, 174, 462). The same compound decreased atherosclerotic lesion development in LDL receptor (LDLR) knockout mice (516). Another LXR agonist, GW3965, induced expression of LXR target genes in the small intestine and also in macrophages and increased reverse cholesterol transport by increasing high-density lipoprotein cholesterol concentration (92). Although LXR ligand treatment reduced atherosclerotic lesion size in LDLR and apolipoprotein E (apoE) knockout animals (240), due to their effects on lipogenesis, the potential benefit of LXR activators in the treatment of atherosclerosis is limited (for details, see sect. VII C).

C. PPARs Bind a Broad Range of Fatty Acids and Diverse Synthetic Ligands

There are three members of the subfamily of PPAR. PPARα is expressed most prominently in the liver and regulates fatty acid oxidation (124). PPARγ, which is expressed in adipose tissue, macrophages, and DCs, regulates lipid uptake and storage (503, 521) and immune responses. The ubiquitously expressed PPARβ/δ is implicated in fatty acid metabolism, mitochondrial respiration, thermogenesis, muscle lipid metabolism, and fibrotype switching (124). They all bind various nonesterified fatty acids, polyunsaturated fatty acids, eicosanoids, or prostanoids and regulate aspects of lipid metabolism in which there is remarkably little overlap in spite of the fact that they all bind to the same response element. A common feature of PPARs is that they are all targets of drug discovery and have clear therapeutic utility. PPARα and PPARγ are the targets of lipid-lowering fibrates (256) and thiazolidinediones (TZDs) used in insulin sensitization (521), respectively. Ligand for PPARβ/δ may be useful in muscle wasting syndromes (376).

In this review we focus on one member of the family, PPARγ, because this has been shown most extensively to act as a modulator of macrophage and DC differentiation and function.

The first endogenous ligand for PPARγ was suggested to be a prostanoid, 15-deoxy-D12,14-prostaglandin J2 (PGJ2) (141, 260, 581). However, the amount of this compound appeared to be too low or nondetectable in tissues for activating PPARγ (417). In macrophages it has been shown that molecules from oxLDL can activate PPARγ. These were identified to be oxidized derivatives of fatty acids, like 9-hydroxyoctadecadienoic acid (HODE) and 13-HODE (325, 371, 461). Recent studies on PPARγ ligand binding revealed that it has a relatively large ligand-binding pocket resulting in the possibility that two ligands can simultaneously occupy the pocket (225). Another unique feature of PPARγ ligand binding is that oxo and/or nitratated fatty acids can be coupled covalently to the receptor generating a more potent activation than noncovalently bound ligands (225, 296, 544).

The essential role of PPARγ has been demonstrated in adipocyte differentiation (30, 57, 270, 445, 519), placental development, and vasculardization (30, 514, 515).

PPARγ deficiency results in embryonic lethality. A disturbance in terminal differentiation of trophoblasts and placental vascularization leads to myocardial thinning and death by E10.0 (30). Tetraploid-rescued mutant exhibited another lethal combination of pathologies, including lipodystrophy, fatty liver, and multiple hemorrhages (270, 445). Those PPARγ null mice that survived to term were deficient for all forms of adipose tissue providing evidence for the fundamental role of PPARγ in adipogenesis (30). In macrophages PPARγ regulates many aspects of lipid/cholesterol metabolism and inflammation, which will be detailed below (77, 371, 437, 520). By coordinating cholesterol uptake and efflux the recep-
tor is a central player in foam cell formation, one of the earliest steps during atherogenesis.

**D. VDR Is a Classical Endocrine Receptor**

In contrast to RXR, LXR, and PPAR, the ligand of VDR was discovered before the cloning of the receptor. The nutritional forms of vitamin D were identified, and their structures were determined during the 1920s and 1930s (reviewed in Ref. 119). 1,25(OH)2D3 (calcitriol), the active form of vitamin D3, was identified and synthesized in the 1970s (143, 205). The existence of the VDR was demonstrated in the 1970s by Brumbaugh et al. (55, 56), while cloning of the cDNA encoding the human and rat VDR was reported in the late 1980s (27, 61, 62). Vitamin D is essential for promoting calcium absorption in the gut and maintaining adequate serum calcium and phosphate concentrations to enable normal mineralization of bone (119). It is also required for bone growth and bone remodeling by osteoblasts and osteoclasts. The classical manifestations of vitamin D deficiency are rickets (in children), osteomalacia, and osteoporosis (in adults) (204). However, a series of experiments demonstrated that VDR is expressed in most tissues in the human body, and vitamin D plays an important role in decreasing the risk of many chronic illnesses, cancers, autoimmune diseases, infectious diseases, and cardiovascular disease (204).

According to the IUPAC-IUB, the term vitamin D should be used as a general term to describe all steroids that exhibit qualitatively the biological activity of cholecalciferol or vitamin D3 (1). In this way, vitamin D refers to many members of a group of secosteroids; these molecules have similar structure to steroids, but one of the rings has been broken. The two major physiologically relevant forms are vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D3 is not found in plants and is present in low abundance in most animal tissues but is a major naturally occurring form of vitamin D in plants (204). Although vitamin D3 has been shown to contribute significantly to the overall vitamin D status in humans (135, 203, 297, 580). The phenotype includes rickets and osteomalacia, elevated levels of and resistance to 1,25(OH)2D3, hypocalcemia, profound secondary hyperparathyroidism, total alopecia, and abnormal skeletal muscle development. Remarkably, depending on the ablated exons of VDR, there are differences in the phenotypes of different VDR−/− mice (e.g., in fertility and life span) (297, 580).

**V. THE BIOLOGY OF RAR AND RXR IN MACROPHAGES AND DENDRITIC CELLS**

**A. Retinoids in Myeloid Development**

RARs are expressed in nearly all hematopoietic lineages (524) and play critical roles in hematopoiesis (248, 250). In myeloid cells, RARα is the dominant isoform; however, RARβ and -γ are also expressed at a lower level (114, 285). RARβ can induce its own transcription (115); however, in myeloid cells the silencing DNA methylation on the promoter of RARβ needs to be first erased for RARβ to be expressed (125). RAR contributes to myeloid differentiation through regulating expression of its target genes involved in the differentiation program. However, the contribution of RAR to early myeloid development and to the granulocyte lineage is more prominent than to the monocytic development (see below). RARα induces C/EBPα (87, 399, 575), CD18 (12, 63), CD38 (128), RTP801, a recently discovered inhibitor of the mTOR pathway (155), interferon regulatory factor-1 (IRF1) (327), and leukocyte alkaline phosphatase (158) and mediates cell cycle arrest, a key phenomenon during differentiation (477, 545). MC-CSF, M-CSF receptor, G-CSF, GM-CSF, and GM-CSF receptor were also reported to be induced by retinoic acid in bone marrow stromal cells (111, 216, 373, 552).
Nonetheless, retinoids have a dual effect on developing cells, either inducing maturation or cell death depending on the cellular context and presence of other factors. In a murine hematopoietic cell line, ATRA inhibited IL-6-induced differentiation by downregulating IL-6 receptor (395).

ATRA not only induces myeloid lineage but can also repress erythroid differentiation by downregulating GATA1, a key regulator of erythroid development (272).

When blood monocytes were cultured in the presence of GM-CSF and ATRA, DC-like cells were generated in terms of morphology, CD1a expression, adhesion, costimulatory molecule expression, and T-cell activation (342).

There is evidence for crosstalk between nuclear receptor pathways involving RAR. Probably through the induction of C/EBPs RAR can induce PPARγ expression and potentiates the effects of PPARγ agonists on assisting macrophage development (499). The PPARγ target gene CD36, a scavenger receptor on macrophages, is also regulated by RAR (189). While ATRA is a more efficient inducer of granulocytic than monocytic development, vitamin D acts oppositely (32, 127, 266, 518). Vitamin D analogs are widely used to induce mononuclear differentiation. Interestingly, a combination of retinoids and vitamin D may be more efficient, suggesting the possibility of a synergistic crosstalk between the two pathways (45, 51, 54, 374).

As far as genetic evidence for the receptors involvement is concerned. RARα and -γ knockout mice display a block in granulocytic differentiation (236, 273). Dominant negative RARα blocks neutrophil development at the promyelocyte stage (235, 525) and switches normal granulocyte/monocyte differentiation to basophil/mast cell development (524). RAR was reported to act as a differentiation checkpoint switch at the promyelocytic stage of granulopoiesis, resulting in granulocytic differentiation (592). Following induction of myelomonocytic differentiation, induction of RARα was observed (593). Retinoic acid (RA) stimulates the maturation of myeloid precursors in cytokine-stimulated CD34+ cells (236). One of the most studied examples of leukemia is acute promyelocytic leukemia (APL). In APL a t(15;17)(q22;q12) chromosome translocation generates a PML-RARα fusion protein that inhibits RAR, resulting in a block of terminal differentiation of granulocytes (236, 241, 553). Promyelocytic leukemia cell lines can be differentiated, and APL patients can be successfully induced into remission with treatment of ATRA (93, 113, 131, 241, 470, 555). The translocation generates a PML-RARα fusion protein, which cannot release histone deacetylase (HDAC) complex at physiological ATRA concentrations leading to the maintenance of repressive chromatin marks and corepressor binding (178, 193, 299). Dissociation of the corepressors NCoR or SMRT from PML-RAR occurs at high doses of ATRA, explaining the biological effect of pharmacological concentrations of RA (178, 180, 193, 299). These results suggest that a failure in HDAC release is the mechanism of APL. This was further supported by the findings on PLZF-RAR fusion protein, which is the result of a t(11;17) translocation (335). Patients with this translocation are resistant to ATRA treatment, since unlike PML-RAR, PLZF-RAR is ATRA insensitive so HDAC and corepressors are not released. Nevertheless, combination of ATRA and HDAC inhibitors can induce relief of repression and admit differentiation. These findings established the role for HDAC inhibitors in clinical therapy (85, 556).

However, the mechanism of PML-RARα-induced repression is more complicated. PML-RARα was also shown to interact with PU.1, a master regulator of hematopoiesis, and the interaction results in the suppression of PU.1, which leads to differentiation block (357). ATRA induces degradation of the PML-RARα and restores PU.1 function. The majority of PU.1 binding sites are in close proximity of variously spaced RAR binding sites, which seemed to be prerequisite for subsequent repression (546).

Besides the HDACs and PU.1, PML-RARα mistargets many chromatin modifiers resulting in the formation of repressive marks, H3K27me3, H3K9me3, and DNA methylation (68, 125, 537). Despite these new findings, it is still elusive what the crucial determinants for PML-RARα binding to a specific region are. It could be the DNA sequence, chromatin structure, interaction with other transcription factors, or the combination of these.

In support of RXR’s involvement, expression of RXRα was reported to correlate with the differentiation stage of myelomonocytic cells showing higher levels in more matured cells (118). RXR-specific agonists were shown to overcome the dominant negative effect of mutant RARα and induce myeloid differentiation (235). RXR agonists independently from the RAR could lead to maturation of APL cells via a crosstalk between RXR and protein kinase A (39). A combination of RAR and RXR activators could induce maturation of myeloid cell lines more efficiently than single agonists (470). However, others report that RAR and RXR have distinct functions in myeloid differentiation by inducing either differentiation or apoptosis, respectively (40, 49, 333). Ligand activation of RARs in HL-60 cells resulted in suppression of BCL-2 expression, whereas ligand activation of both RARs and RXRs triggered the selective accumulation of tissue transglutaminase in the apoptotic HL-60 cells (367, 369, 370). RXRβ was also shown to perturb myeloid differentiation when a dominant negative mutant was expressed in mice (493). Nevertheless, myeloid-specific deletion of RXRα had no major consequences on hematopoiesis (439).
NUCLEAR RECEPTORS IN DENDRITIC CELLS AND MACROPHAGES

Therefore, probably due to the extensive combinatorial possibilities of RAR regulation and the promiscuity of RXR, the deconvolution of the exact role of RARs in myeloid cell differentiation is far from over.

B. Retinoids in Monocyte/Macrophage Function

The availability of natural and synthetic ligands for RAR and RXR made it possible to generate a vast array of data regarding monocyte/macrophage function. However, only a fraction of these findings have been ascribed to receptor activation using more extensive pharmacological and/or genetic approaches. Therefore, it is quite possible that some of the effects are not receptor-dependent or do not require the receptor’s genomic activity.

The mobility of monocytes/macrophages is affected by retinoids. 9-cis RA was shown to induce monocyte chemoattractant protein expression in human monocytic leukemia cells (594). G-CSF and a novel RARα-specific agonist, VTP195183, were shown to synergize and enhance the mobilization of hematopoietic progenitor cells from the bone marrow (196). The effect of retinoids on cytokine production is also controversial in the literature.

There are pieces of evidence for both a pro- and anti-inflammatory role. The pieces of evidence for an anti-inflammatory role are the following: ATRA was shown to inhibit TNF and nitric oxide production in peritoneal macrophages (334). In addition, ATRA enhanced the production of IL-10 and nitric oxide production in peritoneal macrophages (334). In a rat model of chronic allograft nephropathy, 13-cis RA was shown to induce monocyte chemoattractant protein expression in human monocytic leukemia cells (594). G-CSF and a novel RARα-specific agonist, VTP195183, were shown to synergize and enhance the mobilization of hematopoietic progenitor cells from the bone marrow (196). The effect of retinoids on cytokine production is also controversial in the literature.

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Another level of the anti-inflammatory effects of retinoids is the attenuation of tissue damage at the site of inflammation. ATRA could induce plasminogen activator inhibitor-2 production in blood mononuclear cells, which inhibited urokinase-induced extracellular proteolysis (346).

In DBA/1J mice with collagen-induced arthritis (CIA), retinoids improved the clinical course of the disease and reduced the production of inflammatory cytokines, immunoglobulin, and chemokines MCP-1, IL-6, IL-12, TNF, total IgG, and IgG1 anti-collagen antibodies (384).

In a rat model of chronic allograft nephropathy, 13-cis RA treatment decreased the number of infiltrating mononuclear cells, their proliferative activity, and synthesis of MCP-1, MIP-1α, IP-10, RANTES, plasminogen activator inhibitor-1, transforming growth factor-β1 (TGF-β1), and collagens I and III (3).

In a model of lupus nephritis in MRL/lpr mice, ATRA reduced lymphoadenopathy and splenomegaly and proteinuria. Although plasma IgG and anti-DNA antibody levels and immunocomplex and complement deposition were not affected, the numbers of infiltrating T cells and macrophages were decreased. Simultaneously, renal TGF-β level was elevated (409). Similar results were obtained when rat glomerulonephritis models were used (286, 457, 458). Both liposomal and free ATRA could decrease the expression of matrix metalloproteinase-9 (MMP-9) and induce its inhibitor, tissue inhibitor of matrix metalloproteinase 1 (TIMP1), in bronchoalveolar lavage cells (142).

There are also pieces of evidence to support a more pro-inflammatory and enhanced antimicrobial response. In a randomized study on rats treated with RA, the severity of tuberculosis infection was attenuated by increasing the numbers of T cells, natural killer cells, and macrophages in the lung and spleen, and the levels of IFN-γ, TNF, IL-1β, and iNOS were higher (573). RARγ-deficient macrophages exhibited impaired inflammatory cytokine production upon TLR stimulation and defective immune response to Listeria monocytogenes (132).

Clearly, a more systematic analysis of RARs contribution to inflammatory paradigms needs to be carried out.

C. Retinoids in Macrophage Metabolism

The role of retinoids in macrophage metabolism is still largely unexplored. A link between RAR, PPAR, and LXR signaling is that CYP27, a mitochondrial P-450 enzyme capable of generating 27-hydroxycholesterol, is under complex regulation by RAR, RXR, and PPARγ (498). The oxidized cholesterol derivative product of CYP27 could activate LXR to induce cholesterol efflux from foam cells, thereby contributing to cholesterol removal from vessel wall and reverse cholesterol transport (148, 231, 498). Costet et al. (98) reported that the RARγ:RXR heterodimer induced the expression of ABCA1 in macrophages. In in vivo studies, ATRA suppressed lesion development induced by Chlamydia pneumoniae in hyperlipidemic C57BL/6J mice while had no effect in uninfected animals (234). The synthetic retinoid AM580 suppressed scavenger receptor expression and macrophage foam cell formation in vitro and prevented atherogenesis in apoE-deficient mice in vivo (510). In carbohydrate metabolism, ATRA induces aldose-1-epimerase (mutarotase), which catalyzes the interconversion of α and β hexoses, essential for normal carbohydrate metabolism and the production of complex oligosaccharides. Through this pathway retinoids could affect energy utilization and synthesis of cell-surface glycoproteins or glycolipids involved in cell motility, adhesion, and/or functional properties (398).
D. Effects of Retinoic Acid on DC Function

There is limited information about the expression of RARs in DCs; however, a few studies indicated that at least some of the isotypes of RAR and RXR proteins can be detected in DCs.

In human monocyte-derived DCs, RARα and RXRα showed the highest expression, and proteins of these receptors were also detected (504). Notably, mouse splenic DCs expressed all RAR isoforms (320). These results suggest that ATRA can exert a receptor-dependent effect on DCs. Before the discovery of ATRA production by intestinal DCs, the effects of retinoids on DC had already been investigated in detail. An early report indicated that in vivo administration of retinol-acetate or 13-cis RA increased the number of DCs and macrophages in spleen; moreover, these cells had an enhanced immunological function (252), suggesting that retinoid signaling had a positive effect on the immunogenicity of DCs. A human study also indicated that local treatment of ATRA on skin promotes the epidermal DC (Langerhans cells) activation because these cells had an enhanced HLA-DR, CD11c, and CD1c expression (338).

Consistent with these results, Geissmann et al. (153) described that in the presence of pro-inflammatory stimuli ATRA further enhanced the maturation of DCs; moreover, it was suggested that the NF-κB activation was responsible for this effect. However, without inflammatory stimulation, ATRA treatment itself elicited programmed cell death in this DC model (153). Our data also indicated that synthetic retinoid-treated DCs regulate a large number of genes and pathways. For example, retinoid signaling coordinately regulates the CD1 gene family expression. Group 1 CD1 genes (CD1a, CD1b, CD1c, and CD1e) were downregulated, whilst group 2 CD1 (CD1d) was upregulated upon ATRA treatment (504). Furthermore, our analysis revealed that the enhanced expression of CD1d leads to an elevated natural killer T-cell activation capacity of these cells. We will further discuss the CD1 gene family regulation on section VIIF. Taken together, these observations suggested that activation of RAR positively modulates the immunogenicity of DCs.

However, other studies reported an opposite effect on RAR activation. An early in vitro study revealed that even low concentrations of ATRA exerted an inhibitory effect on spleen-derived mouse DCs because these cells had an impaired T-cell stimulatory capacity (35). In line with this finding, ATRA inhibited in vitro differentiation and maturation of DCs developed from human cord blood monocytes with impaired IL-12 and increased IL-10 production (513). A recent report also indicated that ATRA-instructed DCs had impaired IL-12 production; moreover, ATRA in vivo might contribute to the IL-12 hypoproductive DC development (543). One study suggested that ex vivo ATRA treatment also confers regulatory T cell inducing capacity of DCs (455). ATRA-treated swine monocyte-derived DCs produce more TGF-β and IL-6; in addition, these authors proposed that these cells could store and release ATRA, and this retinoid signal also contributed for polarization of T-cell development (455). In mouse splenic DCs, zymosan plus retinol treatment turned on endogenous ATRA production, and it was suggested that the endogenous ATRA acted in an autocrine manner to suppress the production of pro-inflammatory cytokines (IL-6, IL-12, and TNF-α) (320). Importantly, in this report, the authors started to explore the potential mechanisms of this anti-inflammatory effect. ATRA production stimulated the expression of SOCS3, and the induction of this repressor protein contributed to the anti-inflammatory effects; moreover, ATRA signaling attenuated the activation of p38 MAP kinase (320) (FIGURE 5). It is still controversial how DC migration is regulated by retinoid signaling. One report suggested that murine bone marrow-derived DCs have enhanced migratory capacity upon ATRA treatment in vivo via the enhanced production of MMP-12 (109). However, it was also described that 9-cis RA or a synthetic retinoid negatively regulated the migration capacity of DCs via the downregulation of CCR7 and CXCR4 chemokine receptors (539).

It is well known that retinoid signaling had an important role for polarization of lymphocyte development (see sect. XB); however, as we have described here, ATRA could also instruct DCs, and this might also contribute for induction of oral tolerance and regulation of the immune response.

VI. THE BIOLOGY OF LXR IN MACROPHAGES AND DENDRITIC CELLS

A. Contribution of LXRs to Macrophage Biology

The role for LXR in macrophages appears to be focused to three broad areas: complex regulation of basic lipid metabolism, modulation of immune responses, and the inhibition of apoptotic pathways (FIGURE 4A).

We would like to note here that the role of LXR and PPARγ in macrophage lipid metabolism will be discussed in detail in section VIIIC.

LXR activity is modulated by the innate immune system, and vice versa, LXR itself can modify cellular immune responses. A key observation showed that mice lacking LXRα were highly susceptible to Gram-positive intracellular pathogen, Listeria monocytogenes infection (237). Bone marrow transplantation experiments revealed that expression of LXRs in hematopoietic cells is required for this effect. An important crosstalk between LXR and TLR signaling was uncovered in cultured macrophages as well as in
aortic tissue in vivo by showing that microbial compounds via TLR3 and TLR4 can inhibit LXR target gene expression and cholesterol efflux in an IFN regulatory factor 3-dependent (IRF3) manner (72). LXR was also reported to exert anti-inflammatory effects by inhibiting the expression of several proinflammatory molecules such as iNOS, cyclooxygenase (COX)-2, IL-6, IL-1β, MCP-1, MCP-3, and MMP-9 in response to bacterial infection or LPS stimulation (71, 238). Therefore, LXR antagonizes inflammatory gene expression downstream of TLR4 and also IL-1β and TNF-α-mediated signaling. On the more mechanistic front, Glass and colleagues (157) demonstrated that a similar mechanism of transrepression exists for LXR as reported previously for PPARγ ligand binding results in SUMOylation of the receptor, which is recruited to the promoters of inflammatory genes and inhibits LPS-induced corepressor (NCoR) clearance. However, similar unresolved issues exist in connection of this model as in the case of PPARγ (see below). This would all point into the direction of LXR being exclusively anti-inflammatory. However, an interesting observation was reported by Fontaine et al. while showing that short-term pretreatment by LXR agonists reduced
the inflammatory response induced by LPS; long-term pre-
treatment, however, resulting in an enhanced LPS response
(140) suggested that the crosstalk between LXR and TLR
signaling is more complex and likely to be context depen-
dent. A difference exists in DCs as well regarding human vs.
mouse cells (see below).

Most recently, the Castrillo and Tontonoz laboratories
jointly demonstrated that LXR is involved in apoptotic cell
clearance (365). Macrophages play a role in the elimination
of apoptotic cells generated by the billions every day. The
uptake is a complex and not well understood process in-
volving “find me” and “eat me” signals (424). Phagocytosis
of apoptotic cells by macrophages is significantly enhanced
upon LXR ligand addition and LXR-deficient macrophages
have a decreased capacity to take up apoptotic cells. Appar-
ently, the lack of the apoptotic cell receptor Mer is respon-
sible for some of these effects, which is identified as an LXR
target gene. In addition, LXR has been shown to directly
regulate RAR (425), and via this some additional factors
got implicated such as the retinoid-regulated enzyme, tissue
transglutaminase which has been linked to apoptotic cell
clearance (367, 509). Defective apoptotic cell uptake can
lead to chronic inflammatory conditions and autoimmune
diseases.

Moreover, LXRα conferred the most protection and the
loss of this receptor correlated with macrophage apoptosis.

As a potential mechanism, Joseph et al. (237) identified
AIM (also known as Spα, API6, and CD5L), a gene that
promotes macrophage survival, as a target of LXRα in
mouse macrophages. AIM also protects macrophages from
apoptosis when exposed to oxLDL, suggesting a role for
LXR as a macrophage survival factor during the develop-
ment of atherosclerosis (237). Valledor et al. (531) reported
similar results by showing the upregulation of antiapoptotic
factors such as Bcl-XL and Birc1a and the inhibition of
proapoptotic elements such as caspases 1, 4/11, 7, 12, Fas
ligand, and DNase I by LXR (531).

**B. LXR and DC Biology**

Expression data suggested that LXR is also expressed in
DCs. Initially it has been established that LXRα is the dom-
nant isoform in differentiating human DCs (156). Further-
more, it was also shown that PPAR-induced maturation of
DCs is impaired together with some in vitro adhesion prop-
erties. This phenomenon was explained by the LXR-depen-
dent regulation of actin-bundling protein fascin (156). In-
terestingly, recent studies also showed that endogenous
LXR ligands are produced by tumors. In turn, these com-
pounds activate LXR and control the migratory capacity of
DCs to the tumor tissues in murine models. Downregulated
CCR7 expression was suggested as an explanation for this
effect. This finding suggests that LXR is being exploited by
tumors to support growth (538). However, there are strik-
ing differences between human and mouse DCs.

Our comprehensive analysis of LXR expression and activa-
tion provided further details on how activation of LXR
induces a differentiation program that effects the response
and phenotype of human DCs (522). This program includes
induction of costimulatory molecules, increases in proin-
flammatory cytokine production, and increases T-cell acti-
vation (FIGURE 5). Circulating blood CD1c+ DCs also re-
sponded to LXR ligand treatment, resulting in the increased
expression of LXR target genes. DCs similarly to other
professional APCs (antigen presenting cells) display a broad
array of TLRs to detect a wide array of pathogenic or dam-
aged self structures. In principle, this results in activation of
NF-κB signaling (226, 431). Importantly, other sensory
mechanisms are likely to participate in signal modulation.
While LPS interferes with LXR signaling in macrophages by
inhibiting the expression of its target genes (72, 238), it
appears that in DCs LPS increases the expression of target
genes. This suggests that LXR signaling is integrated in the
TLR signaling pathway by a context- and cell type-specific
manner. It is possible that DCs induce an increased LXR
response when exposed to specific TLR activators, provided
a ligand is available. This might be a hint about the exis-
tence of a potential mechanism for pathogene elimination
by an enhanced inflammatory response. The source and
origin of such ligand(s) are unknown yet. However, the fact
that the production of LXR activating lipids from tumors
could be detected (538) suggests that the inflammatory en-
vironment has a role in determining the receptor’s activity
also in vivo. If put together, the data obtained in macro-
phages and DCs strongly suggest the existence of an intrigu-
ing and so far largely unexplored crosstalk between TLR
and LXR signaling. This also means that LXR activation
may blunt or enhance host defense depending on the con-
text. How cellular or immune context influences this cross-
talk remains to be determined.

**VII. THE BIOLOGY OF PPARγ IN
MACROPHAGES AND DENDRITIC
CELLS**

**A. PPARγ in Myeloid Development**

PPARγ was shown to influence myeloid development (371,
499, 520). Activation of the receptor by natural or synthetic
agonists results in elevated expression of monocytc/macro-
phage markers such as CD14, CD36, CD11b, CD11c, and
CD18 on myeloid leukemia cells. PPARγ seems to regulate
later stages of the development favoring monocytc develop-
ment; in addition, expression of the receptor correlates
with the developmental stage within the monocytic lineage
with highest level in the macrophages (499). Several groups
succeeded to differentiate macrophages from PPARγ-defi-
cient embryonic stem cells, indicating that PPARγ is dis-
persable for monocytc differentiation (76, 347). Its func-
tion is rather a modulator of the immune and metabolic
functions of the macrophages.
B. Anti-inflammatory Role of PPARγ in Monocyte/Macrophage

When PPARγ was identified in mouse thioglycollate-elicited macrophages, its activators were shown to exert potent anti-inflammatory effects (437). Originally, they were shown to inhibit macrophage responses to pro-inflammatory stimuli such as LPS or IFN-γ. Natural and synthetic PPARγ agonists were shown to inhibit production of pro-inflammatory molecules, such as iNOS, MMP-9, and SR-A (437). Similar effects were reported from human monocyte-derived macrophages (233), which led to the conclusion that PPARγ agonists may act as potential anti-inflammatory drugs. Over the last 10 years, PPARγ agonists have been shown to exert anti-inflammatory effects at multiple levels. For example, PPARγ agonists were shown to inhibit the production of many proinflammatory cytokines like TNF, IL-6, IL-1β, IL-12, and gelatinase B (17, 89, 233, 293, 437, 438, 489, 561). Furthermore, PPARγ inhibits macrophage migration and recruitment by repressing the transcription of chemoattractant molecules such as MCP-1 and its receptor CCR2 (25, 31, 82, 188, 469). PPARγ agonists can also induce the production of an anti-inflammatory cytokine, IL-10 (258). Moreover, PPARγ suppresses TGF-β production indirectly by the phosphatase and tensin homolog deleted on chromosome 10 (PTEN) (280, 281).

Although several potential mechanisms for the anti-inflammatory function have been assigned, the exact mechanism is still elusive. The most accepted theory suggests that PPARγ agonists interfere with other transcription factors involved in pro-inflammatory signal transduction pathways, such as AP-1, STAT-1, NF-κB, and NFAT. This mechanism requires a physical interaction between PPARγ and the other transcription factor and subsequent inhibition, a process called transrepression (402, 436). Transrepression is clearly different from the canonical action of nuclear receptors, when they bind to cognate DNA sequences and induce or repress their target genes directly. The molecular mechanism of transrepression is poorly defined; however, it was suggested that there is a ligand-dependent SUMOylation of PPARγ that targets the receptor to corepressor complexes on inflammatory genes’ promoters. This prevents the ubiquitination/proteasome-mediated degradation of repressor complexes, which are required for gene expression (401). This model, however, does not explain how ligand-bound PPARγ can exert two opposite reactions simultaneously: dissociation of the corepressor complex from the receptor to induce target gene expression and recruitment of the receptor to corepressor complexes associated with other transcription factors to inhibit proinflammatory gene expression. Importantly, the negative regulatory role assigned to PPARγ is not a direct transcriptional effect of the receptor but the consequence of the failed induction of inflammatory genes by other transcription factors activated upon proinflammatory molecules, e.g., LPS (293, 402). Key in vivo genetic evidence is still lacking to support this scenario. Another possible mechanism is the sequestration of essential coactivators when their levels are limiting. Activation of PPARγ upregulates PTEN, which inhibits phosphatidylidyinositol 3-kinase-mediated signaling cascades affecting cell migration, survival, and proliferation, providing a further possibility for suppression of inhibitory reaction (403).

Another intriguing possibility to exert anti-inflammatory roles comes from the link between PPARγ and the anti-inflammatory cytokine IL-4. Initially, Glass and colleagues (217) showed that IL-4 induced PPARγ and an enzyme, 12/15-loxyxygenase, capable of ligand production. However, the amount of this compound (15d-PGJ2) appeared to be low in tissues for activating PPARγ (417). Recently, Odegaaard et al. (387) reported that disruption of PPARγ in myeloid cells resulted in impaired alternative macrophage activation and claimed PPARγ to be requisite for the differentiation of alternatively activated macrophages. This might not be generally applicable, because in another mouse strain, the receptor appeared to be dispensable (322).

We recently showed that PPARγ is dispensable for alternative macrophage activation per se and IL-4-induced gene expression regulation; however, we found that IL-4 and its effector transcription factor STAT6 is required for PPARγ activity in alternatively activated macrophages. We suggested an interesting mechanism for the crosstalk between PPARγ and STAT6 by showing that STAT6 acts as a licensing factor for PPARγ. STAT6 physically interacts with PPARγ by binding the enhancers of PPAR target genes and induces PPARγ-mediated transcription (497).

### FIGURE 5. Molecular events regulated by nuclear receptor ligands alter the antigen presenting function of dendritic cells. Events leading to altered lymphocyte activation are depicted. Note that many genes regulated similarly in macrophages and DCs are not indicated. A: TLR2 signaling induces splenic DCs to express retinoic acid metabolizing enzymes and IL-10 and stimulate Tregs. All-trans-retinoic acid (ATRA) induces suppressor of cytokine signaling-3 expression (SOCS3), which suppresses the activation of p38 and proinflammatory cytokines for details see text. B: the upregulation of Cd1d by RAR agonists leads to an enhanced iNKT cell expansion. PPARγ can turn ATRA by inducing the expression of retinol and retinal metabolizing enzymes. Therefore, the effects of PPARγ and RAR agonists are similar in many aspects. The upregulation of cathepsin D (CatD) lysesosomal protease and CD1d leads to an altered lipid antigen presentation and enhanced iNKT cell expansion. CD1d is regulated by RAR, while CatD is regulated by both RAR and PPARγ in human monocyte-derived DCs. C: DCs reprogrammed with LXR agonist have an increased response to TLR signaling and an augmented cytokine response resulting in proinflammatory T-cell responses. D: activation of VDR leads to a tolerogenic DC phenotype, characterized by decreased expression of CD40, CD80, and CD86 costimulatory molecules, low IL-12, and enhanced IL-10 and CCL22 secretion. The expression of antigen-presenting molecules is also lower, while the level of inhibitory receptors, such as ILT3, is elevated.
It is worth mentioning that PPARγ agonists can exert receptor-independent effects as shown when PPARγ knock-out embryonic stem cells were differentiated to macrophages, which still elicited anti-inflammatory reactions in response to PPARγ agonists (76, 347). Welch et al. (561) also found that PPARγ macrophage-specific conditional knockout mice still show anti-inflammatory effects upon treatment with PPARγ activators. They suggested that some of these effects could go through another nuclear receptor, PPARδ, since at high concentrations PPARγ agonists can activate PPARδ as well (561). It was also reported that 15d-PGJ2 can repress NF-κB activity PPARγ-independently via inhibiting IkB kinase and/or by alkylating p50–65 NF-κB heterodimers (70, 489). Another PPARγ-independent mechanism involves the induction of suppressor of cytokine signaling (SOCS) 1 and 3, which results in reduced phosphorylation of STAT1, STAT3, Janus kinase 1 (JAK1), and JAK2 in activated astrocytes and microglia (232, 400).

Remarkably, when the transcriptomes of macrophages were analyzed with DNA microarrays, only a surprisingly small number of regulated genes were found upon PPARγ activation (561). The question of macrophage PPARγ response has been addressed recently by us showing the requirement of IL-4 and STAT6 for the induction of PPARγ target genes (497).

PPARγ and/or its activators have been implicated in several studies on inflammatory diseases. Several human diseases and mouse models have been analyzed so far, and PPARγ agonists were tested whether they inhibit disease progression. Here, without going into the details, we refer to a previous review where we summarized the effects of PPARγ activators in airway and intestinal inflammation, autoimmune encephalomyelitis, myocarditis, arthritis, and dermatitis (500). For a more detailed overview of PPARγ in airway inflammation, we refer to earlier reviews by others (467, 481, 554).

Little evidence has been provided that these effects are really PPARγ-dependent and not nonspecific consequences of the agonists. PPARγ knockout models have not been widely involved in such studies, and the mechanisms of the anti-inflammatory effects have also not been addressed in sufficient depth. However, there are a few reports on PPARγ-deficient animals. PPARγ-deficient heterozygous mice displayed enhanced susceptibility to 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis (123).

5-Aminosalicylic acid (5-ASA), an anti-inflammatory drug widely used in the treatment of inflammatory bowel disease, had beneficial effects on colitis only in wild-type but not in PPARγ heterozygous mice. It was shown that 5-ASA binds to and activates PPARγ and exerts its anti-inflammatory effects through the receptor (446). Targeted disruption of PPARγ in macrophages (469) or colon epithelial cells (2) increased susceptibility of mice to dextran sodium sulfate-induced colitis.

C. Atherosclerosis: Role of LXR and PPARγ in Macrophage Metabolism

As detailed above, several lines of evidence support a central role for macrophage-mediated inflammation in the pathogenesis of metabolic diseases. Macrophage infiltration and the formation of foam cells is a prominent feature during atherogenesis (FIGURE 6). Recently, both proinflammatory classically activated and anti-inflammatory alternatively activated macrophages were shown to be present in fatty streaks (52). PPARγ was shown to be expressed in lesion macrophages (437, 520), and a weak correlation was found between the expression of PPARγ and alternative macrophage markers (52). However, further studies are required to clarify the effect of inflammatory milieu on macrophages and PPARγ activity in the progression of the atherosclerotic plaque.

The function of PPARγ in foam cells has been characterized in detail. PPARγ expression in macrophages could be further increased with oxLDL (371). PPARγ had a key role in the regulation of oxLDL uptake into macrophages/foam cells by inducing its direct target gene, the scavenger receptor CD36. Two components from the lipids in oxLDL, 9-HODE and 13-HODE, were identified as endogenous activators and bona fide ligands of PPARγ (225, 371). These results suggested the following model: macrophages internalize modified or oxLDL via scavenger receptors (e.g., CD36), which unlike LDL receptor are not downregulated by high intracellular cholesterol levels. On the contrary, oxLDL can further increase the expression of CD36 by providing ligands for PPARγ and via this positive feedback potentiates its own uptake. Oxidative modification of the LDL particle endows it with the ability to bind scavenger receptors and at the same time converts a component of the particle, linoleic acid, into an effective activator of PPARγ, 9- and 13-HODE.

OxLDL also induces SR-A expression via a PPARγ-independent way. This regulatory circuit was termed gammacycle and suggested a novel mechanism how oxLDL contributed to foam cell formation and atherosclerosis. These findings suggested the existence of a vicious cycle (positive-feedback loop) leading to atherosclerosis and assigned PPARγ a potential pro-atherogenic role. However, synthetic agonists of PPARγ, the TZDs, are used in treatment of type II diabetes. To dissect the in vivo contribution of PPARγ to the process of atherosclerosis, Chawla et al. (77) generated mice lacking PPARγ in the macrophages. Due to the fact that PPARγ total knockout is embryonic lethal, chimeric mice were used for bone marrow transplantation into irradiated animals. Transplantation of PPARγ null bone marrow into LDL recep-
tor knockout animals resulted in significant increase of the atherosclerotic lesion size. Additionally, Glass and co-workers (293) reported that TZDs inhibited the development of atherosclerosis in LDL receptor-deficient male mice. Similar results were shown by Z. Chen and colleagues in apoE knockout mice, another murine atherosclerosis model (83). Targeted disruption of the PPARγ gene from macrophages resulted in reduced total plasma and HDL cholesterol levels, and cholesterol efflux was significantly decreased from macrophages elicited by thioglycolate in mutant mice (14). Based on these observations, PPARγ can be considered an anti-atherogenic molecule (FIG. 6). Seeking the molecular mechanism assigned a central role for PPARγ and LXR in regulating cholesterol uptake and efflux during foam cell formation. These generated a contradiction to the previous observations on PPARγ-induced oxLDL uptake. This was resolved by showing that activation of PPARγ not only results in cholesterol uptake, but it can also increase cholesterol efflux from the macrophages. It was shown that PPARγ could directly induce transcription of the oxysterol receptor LXR (77). PPARγ induces ABCA1, ABCG1 expression, and cholesterol removal from macrophages through a transcriptional cascade via LXRα. The promoter of the ABCA1 gene was analyzed and while LXR:RXR could activate it, PPARγ:RXR heterodimer could not (77). They compared PPARγ- and LXR-induced cholesterol efflux and found that agonists of both nuclear receptors induced cholesterol efflux, and the combination of the ligands was additive. ABCA1 and ABCG1 are members of the ATP-binding cassette family of transporter proteins and are highly expressed in lipid-loaded macrophages (536). Mutations in ABCA1 gene result in Tangier disease, a disease characterized by marked cholesterol accumulation in macrophages and impaired cholesterol efflux from fibroblasts (48, 53, 449). Several studies reported that LXRs mediate cholesterol efflux by inducing cholesterol transporters ABCA1, ABCG1, and ABCD1. However, the role of SREBP-2 in cholesterol efflux remains controversial (537, 538). From the above observations, it is evident that the coordinated regulation of cholesterol uptake and efflux is crucial for maintaining the lipid homeostasis in cells. Through this fine control, cells can properly respond to environmental changes and maintain their biological functions. (FIG. 6)
ABCGL, and later ABCG5 and ABCG8 (99, 435, 535, 536). Cholesterol efflux from macrophages is likely to be anti-atherogenic. By showing that LXRα is a direct target gene of PPARγ, activators of PPARγ were considered as inducers of LXR-dependent cholesterol efflux. In this modified gamma cycle, activation of PPARγ results in the expression and activation of LXR, which in turn induces ABC transporter expression and leads to cholesterol efflux from macrophages. However, the function of such cycle depends on the presence of endogenous LXR activators. Since both PPARγ and LXR could be activated by lipid components of oxLDL, it was hypothesized that these nuclear receptors composed a transcriptional cascade that regulated macrophage response to oxLDL (275). The fact that LXR is activated in macrophages exposed to acetylated LDL (275), which is not supposed to contain oxidized sterols, suggests the existence of alternative ways to activate LXR. LXR:RXR heterodimers were originally identified as mediators of an alternative retinoid signaling pathway (564, 565) showing that LXR:RXR heterodimer is highly permissive and can be activated from either the RXR side by retinoids or the LXR side by oxysterols (435). Due to the fact that PPARγ:RXR heterodimer is also permissive, one obvious possibility for the dual activation of the two dimers evokes from the RXR side. The other possible mechanism is the generation of an LXR agonist. A number of oxysterols were identified as potential endogenous ligands for LXR (230, 231, 284, 404). As Szanto et al. (498) showed, both mechanisms are utilized in the macrophages. Retinoids via RAR and RXR and oxidized lipids via PPARγ can induce the transcription of a p450 enzyme, CYP27, which generates 27-hydroxycholesterol, an endogenous LXR activator (498) (see above). CYP27 is a mitochondrial enzyme involved in the alternative bile acid synthesis pathway (19, 65, 414, 447, 448) and is expressed in addition to the liver in the lung and also in macrophages (26, 46). It has been also associated with atherosclerotic lesions (102, 219). A mutation in the enzyme results in a rare sterol storage disease, cerebroretinoid xanthomatosis, characterized by xanthomas in tendons and also in the CNS leading to ataxia, spinal cord paresis, neurological dysfunctions, normolipidemic xanthomatosis, and accelerated atherosclerosis (47, 64, 341). Taken together, oxLDL not only provides ligand for PPARγ activation, but PPARγ also induces LXR function via two mechanisms: 1) it induces the receptor and 2) it induces an enzyme that generates ligand for it. A recent discovery of LXR regulation of Inducible Degrader of LDL receptor (Idol) further reinforces the notion that activation of LXR leads to increased efflux by also inhibiting further uptake (587). The consequence of the uncovered network is increased cholesterol turnover in the macrophages with induction of reverse cholesterol transport. Any disturbance in the activity of the participants or overload of this system results in cholesterol accumulation and contributes to atherosclerosis.

D. Role of Myeloid Cell PPARγ in Insulin Resistance and Obesity

Recently, inflammation and inflammatory cells have been described to be key regulators not only in atherosclerosis, but also in obesity and insulin resistance (212). Due to the fact that macrophages are located throughout the body including interfaces, they provide a potential role in the interplay between metabolic and inflammatory processes.

Chronic inflammation is now considered as a general phenomenon in obesity and diabetes mellitus. Infiltration of adipose tissue with classically activated macrophages has been recently described in obese conditions (243, 244, 472, 560, 571). However, the presence of alternatively activated macrophages in adipose tissue and liver reduces tissue inflammation, increases oxidative metabolism, and improves insulin resistance in obese mice (386).

It was suggested that overnutrition leads to the recruitment of inflammatory macrophages to the adipose tissue, changing its original alternatively activated phenotype to classically activated one. This then further promotes obesity and leads to insulin resistance. As reported by Odegard et al. (387), macrophage-specific PPARγ knockout mice were predisposed to development of diet-induced obesity, insulin resistance, and glucose intolerance. Furthermore, they reported the downregulation of genes in oxidative phosphorylation in both skeletal muscle and liver, which led to decreased insulin sensitivity in these tissues (387). Hevener et al. (197) reported similar results: macrophage-specific PPARγ knockout animals have glucose intolerance and insulin resistance in the skeletal muscle and liver accompanied by increased expression of inflammatory mediators. Although these reports suggest important roles for macrophages in the development of insulin resistance and obesity, they cannot be generally applicable, since Marathe et al. (322) found PPARγ−/−, δ−/−, and LXRs dispensable for alternative activation and also their loss in macrophages did not exert major effects on obesity or glucose tolerance. Similarly, rosiglitazone improved glucose tolerance in mice lacking macrophage PPARγ, suggesting that other cell types than macrophages are the primary mediators of the antidiabetic effects of PPARγ agonists (322).

E. PPARγ Expression in DCs

IL-4 is an important factor for the alternative activation of macrophages; moreover, this cytokine positively regulates PPARγ expression in macrophages (217). Interestingly, IL-4 is a frequently used cytokine for the ex vivo generation of human DCs from peripheral blood monocytes (452).
Remarkably, a global gene expression profile analysis revealed that PPARγ was one of the highly upregulated transcription factors during IL-4/GM-CSF-driven monocyte-derived DC differentiation (279). The elevated expression of PPARγ was confirmed with more detailed transcript and protein analyses. Gosset et al. (170) described that PPARγ RNA and protein are highly upregulated upon human monocyte-derived DC differentiation, and later, others also observed robust induction of PPARγ upon DC development from monocytes (378, 502). In addition, our immunohistochemical analysis revealed that in human lymphoid tissue (tonsil) some of the S100 positive cells were also PPARγ positive, suggesting that in vivo, at least a subset of DCs also express PPARγ (502). PPARγ expression in DCs is not human specific, since CD11c splenic DCs also express this receptor (137) and PPARγ protein was also detected in murine bone marrow-derived DCs (186).

RXRα, the dimerization partner for PPARs, was detected in monocyte-derived DCs (504), and it was reported that RXR agonist 9-cis RA has a similar phenotype as PPARγ-specific ligands (585), suggesting the PPARγ-RXR heterodimer can be activated from both sides of the heterodimer. Taken together, these observations revealed that various subsets of DCs expressed PPARγ, suggesting a role in modulation of differentiation and/or activation of DCs.

F. Modulation of the Immunogenicity and Lipid Metabolism of DCs by Activation of PPARγ

PPARγ can be activated with synthetic PPARγ agonists in DCs (for example, with rosiglitazone or troglitazone) as shown by the induction of several generic PPARγ target genes (CD36, FABP4/aP2) after ligand administration (502, 503). Much effort has been applied over the past few years to characterize the effects of the activation of PPARγ in DCs. Most of these studies investigated the immune function of DCs by assessing their cell surface phenotype, cytokine production, and T-cell activation capacity. First, it was suggested that PPARγ activation did not change the immunogenicity (T-cell activation capacity) of murine splenic DCs, although these cells had impaired IL-12 production (137). A similar finding was obtained when the same investigators studied the effects of PPARγ ligand on human monocyte-derived DCs. PPARγ agonist-treated DCs had impaired IL-12 production; moreover, expression of IP-10 and RANTES chemokines were also downregulated, while several cytokines were unaffected (IL-6, IL-10, IL-1β, and TNF-α) (170). In addition, PPARγ agonists positively regulated CD86 expression whilst CD80 expression was downmodulated. These findings suggested that PPARγ activation modulated the polarization of DC-dependent immune response. The authors suggested that activation of PPARγ pathway in DCs favors the differentiation of naive T cells into type 2 cytokine-producing T lymphocytes (Th2 cells), since Th1 polarizing factor IL-12 profoundly decreased; in addition, the diminished production of IP-10 and RANTES may participate in the preferential recruitment of Th2 rather than Th1 lymphocytes. Notably, in these studies, cells were challenged with PPARγ activators during the maturation of DCs; however, some other reports investigated the ex vivo effect of PPARγ ligand during the course of DC development from monocytes. In these cases, ligand treatment had a profound inhibitory effect on the immunophenotype of DCs. Nencioni et al. (378) described that activation of PPARγ affected DC immunogenicity and reverted them to a less stimulatory mode. They confirmed the impaired IL-12 production, diminished CD80, and enhanced CD86 expression; however, several other cytokines (IL-6, IL-15, TNF) were also downregulated in the presence of PPARγ ligand (378). In addition, these cells expressed less CD40 and CD83, suggesting that PPARγ-activated DCs conferred an impaired immunogenicity. Consistent with these findings, PPARγ ligand treatment severely blunted the T-cell activation capacity of these cells (378). This report also described that PPARγ-instructed DCs expressed less CCR7, an important chemokine for DC migration. In line with this finding, murine Langerhans cells had an impaired migration capacity upon in vivo administration of PPARγ agonist (22). Interestingly, a reduced expression of CCR7 was also detected in bone marrow-derived, ovalbumin-pulsed DCs, and PPARγ activation also decreased the migratory capacity of these cells (186). In addition, in vivo administration of the PPARγ agonist pioglitazone reduced the tipDC (TNF/iNOS producing inflammatory DC) accumulation in lung upon H5N1 influenza A virus infection (16). It is possible that a PPARγ-dependent impaired migration is responsible for the reduced recruitment of this DC subtype to the airway. The diminished DC immunity was also described in ex vivo murine DC models. Bone marrow-derived, PPARγ ligand-treated DCs also had an impaired T-cell activation capacity (262). Importantly, the negative effects of PPARγ ligand on DC priming were not observed in PPARγ knockout DCs, suggesting that these repressive effects were receptor dependent (262).

In the macrophage section, we have already discussed in detail the potential mechanisms of the anti-inflammatory effects of PPARγ activators. It was suggested that PPARγ interfered with the NF-κB pathway, which is important for the activation/maturation of DCs (397). Indeed, PPARγ agonist-treated, monocyte-derived DCs had diminished nuclear localization of c-Rel, a component of NF-κB (23, 378). In addition, it seems that the MAP kinase pathway was also affected: PPARγ-instructed DCs showed impaired phosphorylation of ERK kinase, and diminished phosphorylation of P38 was detected upon TLR4-dependent maturation (23). NF-κB binding and impaired MAP kinase signaling pathway was also described in murine bone marrow-derived DCs, suggesting that it is a general repressive mechanism in DCs (262). Interestingly, activation of PPARγ leads
to the upregulation of the coinhibitory molecule B7H1, and this inhibitory signal contributed for impaired activation of CD8+ T cells (263), suggesting that this mechanism is also part of the anti-inflammatory effects of PPARγ. In summary, several lines of evidence suggested that activation of PPARγ has an adverse effect on DC immunity, maturation, and migration.

However, in addition to these negative effects on DC development, we and others identified several pathways and target genes, which were positively regulated by PPARγ agonists. PPARγ-activated monocyte-derived DCs had an enhanced FITC-dextran uptake capacity (23, 502). PPARγ ligand treatment resulted in increased mannose receptor expression in mouse DCs, which consequently led to enhanced mannose receptor-mediated endocytosis of soluble ovalbumin antigen (263). CD36 was one of the first identified PPAR targets in DCs (170, 378), and this receptor is implicated in antigen uptake.

The physiological role of this scavenger receptor on DCs is still controversial. It was suggested that CD36 had an important role for apoptotic cell uptake and cross presentation of antigens to cytotoxic T cells in human DCs (15). However, the CD36-deficient animal model revealed that CD36 is not essential for cross presentation of cellular antigens in vivo (59, 463). The other well-established PPARγ target is aP2 (alternative name FABP4). This adipose-specific fatty acid binding protein is a very abundant protein in fat cells (192). Remarkably, in monocyte-derived DCs, this transcript showed the highest upregulation upon PPARγ ligand treatment (502); in addition, the protein level was also strongly induced in PPARγ-activated DCs (505). The potential role of this lipid binding protein in DCs is poorly characterized; however, bone marrow-derived DCs obtained from FABP4/aP2-deficient animals had impaired cytokine production (IL-12, TNF) and diminished T-cell activation capacity, suggesting that at least a low level of this protein positively regulated DC immunogenicity (443). Interestingly, one member of the ABC transporter family was directly regulated by PPARγ in monocyte-derived DCs. Our data revealed that PPARγ induced the expression of ABCG2 in differentiated human DCs; moreover, we identified a specific enhancer region containing three PPAR response elements in the upstream region of the ABCG2 gene (506). The ABCG2 protein is highly expressed in the plasma membrane of several drug-resistant cell lines, where it has been shown to extrude anti-tumor drugs (454); consistent with these findings, our results indicated that PPARγ-instructed DCs had an enhanced xenobiotic extrusion capacity (526).

Interestingly, besides the upregulation of individual genes, our global gene expression profile analyses revealed that a gene cluster and a few signaling pathways were also modulated in PPARγ-instructed DCs. We found that the lipid antigen presentation capacity of DC appears to be impacted by PPARγ activation via the coordinate regulation of CD1 proteins. CD1 proteins are MHC class I-like molecules that present lipid antigens to T cells; thus CD1s play an important role in acquired immunity. These proteins are divided into two groups: group 1 comprises CD1a, CD1b, CD1c, and CD1e and presents antigens derived from pathogenic microbes; group 2 contains only CD1d that is considered to present self-glycolipids, and this protein is necessary for generation and expansion of iNKT (invariant natural killer T) cells (36, 254). We detected a reduced expression of CD1a, -b, -c, and -e and an enhanced expression of CD1d in human PPARγ-activated monocyte-derived DCs (502). The functional consequence of the elevated expression of CD1d was the selective expansion of iNKT cells (502). Recently, further mechanistic insights have been gained into this process by demonstrating that a lysosomal aspartyl protease, cathepsin D, is also regulated by PPARγ and also by RAR, and it contributes to the lipid antigen presentation process by cleaving ProSaposin and thereby facilitating lipid antigen processing (375) (FIGURE 5).

Our global gene expression analysis also indicated that PPARγ activation might modulate the lipid metabolism of DCs. A time course experiment of PPARγ ligand treatment revealed that genes involved in lipid metabolism comprised the primary class of transcripts changed in early stages of DC differentiation, while genes linked to immune responses were altered at later phases of DC development. These results suggested that PPARγ primarily modulated the lipid metabolism/transport in DCs and immune response-related genes are indirectly regulated (505). It is still unexplored how the two pathways are connected in DCs, but in macrophages it was suggested that lipid metabolism and immunity are interconnected (533).

In summary, activation of the PPARγ pathway in DCs has detrimental effects; it is important to emphasize that most of these effects are receptor dependent, since these could be reverted by a PPARγ antagonist (GW9662) (21, 502). More importantly, conditional knockout data also revealed the receptor dependency (262). In addition, we found that several genes showed impaired induction/repression in PPARγ-activated DCs obtained from patients harboring mutated PPARγ receptors (11, 505). In the future it would
be important to characterize the effects of PPARγ activators on DC development and function in vivo.

G. Natural/Endogenous Ligands for Modulation of the PPARγ Pathway in DCs

In the previous section we have demonstrated that activation of PPARγ exerted detrimental effects on differentiation and immunogenicity of DCs. Most of these studies used high-affinity, synthetic PPARγ activators; however, it is unclear whether the natural/endogenous PPARγ ligands can regulate these pathways in DCs or not. It is generally accepted that high concentration of polyunsaturated fatty acids, oxidized fatty acids, oxidized phospholipids, and several prostanoids can serve as natural ligands for PPARγ. Interestingly, some of these lipid mediators also regulate the immune response; more importantly, migratory DCs might be exposed or even produce these lipid mediators. The first lipid mediator was prostaglandin D2 (PGD2) as potential activator of PPARγ in DCs. Angeli et al. (20) described that PGD2 inhibited the migration of Langerhans cells. Synthetic PPARγ agonists had similar inhibitory effects (21), suggesting that it might be a receptor-dependent effect. PGD2 binds to cell surface receptors, and it is unlikely to be a PPARγ-specific ligand. However, 15-deoxy PGJ2 (15d-PGJ2), the dehydration end product of PGD2, is a high-affinity ligand of PPARγ receptor (141, 260). Interestingly, administration of 15d-PGJ2 had similar effects as troglitazone or rosiglitazone (well-established PPARγ activators) on DC differentiation and activation (378). However, it is worth mentioning that this lipid compound had some PPARγ-independent effects (489); moreover, the physiological role of this molecule is still debated due to its low concentration in vivo (417).

oxLDL contains several lipid species, which were described as PPARγ ligands (110, 371). DCs might also be engaged with oxLDL, and several reports documented the effects of oxLDL on DC development. We found that oxLDL indeed induced the expression of PPARγ targets (FABP4/aP2, CD1d); however, this effect was much less profound as a synthetic PPARγ activator (502). Some of the regulated genes were also induced by retinoids; thus it is possible that upregulation of CD1d was mediated by the retinoid content of the oxLDL (504). On the other hand, oxLDL promoted the maturation of DCs (101), and probably this is a PPARγ-dependent signaling pathway since the pro-inflammatory lysophosphatidylcholine (LPC) could mediate this effect through the activation of a G protein-coupled receptor (100). Taken together, these results demonstrate that complex lipids like oxLDL have detrimental effects on DC activation and probably multiple pathways are involved.

There are several other lipid compounds that were considered as PPARγ agonists in DCs. For instance, pollen grains contain pollen-associated lipid mediators; among these, E1 phyt propane (PPE1) was suggested to be an activator of PPARγ (160). It is worth mentioning that this compound had a PPARγ-dependent and -independent effect on DCs (160). Polyunsaturated fatty acids are natural ligands of PPARγ. Consistent with this notion, doxosahexaenoic acid, a polyunsaturated fatty acid, had a similar effect as synthetic PPARγ activators on DCs (586). The authors assumed that these effects were mediated by activation of PPARγ. However, this compound also binds RXR, suggesting that it can activate both partners of the PPAR:RXR heterodimer (586). Interestingly, our data indicated that the serum itself could modulate the activation stage of PPARγ in developing DCs. When monocyte-derived DCs were differentiated in human serum instead of fetal bovine serum, an elevated expression of several PPARγ targets (FABP4/aP2, ABCG2) was detected, and this could be reverted with PPARγ antagonist, suggesting this to be a receptor-mediated process (502, 506). A more systematic gene expression profile analysis revealed that in human serum-cultivated DCs, a similar set of transcripts was upregulated as in synthetic PPARγ ligand-treated cells, indicating that a high affinity or high concentration of ligand might be present in or generated from human serum (505). The nature of such putative ligand is poorly defined; however, one study suggested that human serum-derived cardiolipin and lysophosphatidic acid might be responsible for the enhanced activation of PPARγ (291).

VIII. THE BIOLOGY OF VDR IN MACROPHAGES AND DENDRITIC CELLS

A. Vitamin D and the Immune System: An Overview

The immunomodulatory role of vitamin D first was demonstrated on T cells and B cells (289, 350, 440). The expression of VDR was documented in many human and murine immune cells, most importantly in macrophages, monocytes, DCs, T cells, B cells, and iNKT cells. Natural and synthetic VDR agonists can modulate a broad range of immune responses in these cells, and immunosuppressive effects of 1,25(OH)2D3 and its analogs also have been documented in in vivo animal models of immune-mediated diseases such as autoimmunity and transplantation. The regulatory role of 1,25(OH)2D3 on T cells and B cells is complex. 1,25(OH)2D3 inhibits T-cell proliferation and downmodulates the expression of IL-2, IFN-γ, and CD8+ T-cell mediated cytotoxicity, while IL-4 production is enhanced (44, 182, 288, 289, 332, 350, 428, 440, 441, 532). 1,25(OH)2D3 blocks the induction of Th1 cell and Th17 responses, while promoting Th2 cell and Treg responses.
In immune cells, 1,25(OH)₂D₃ regulates the gene expression by various molecular mechanisms. CYP24A1, CAMP, PPARδ, and ALOX5 are upregulated directly by VDR:RXR (79, 130, 167, 480). Some immune function-related genes, e.g., IER3 and RelB, are downregulated directly via negative response element (126, 221). Ligand-bound VDR can also act by antagonizing the “inflammatory” transcription factors like NFATp/AP-1 and NF-κB, which results in decreased expression of IL-2 and IL-12, respectively (106, 511). 1,25(OH)₂D₃ can also regulate gene expression by indirect mechanisms, by alteration of the level of enzymes, transcription factors, and coregulators (532).

B. Effect of 1,25(OH)₂D₃ on Monocytes/ Macrophages

The immunosuppressive effects of vitamin D on monocytes and macrophages are intensively investigated and well documented. 1,25(OH)₂D₃ inhibits the expression of all three subtypes of MHC class II antigens (HLA-DR, -DP, and -DQ) (572). VDR agonists exert strong immunosuppressive effects on IFN-γ-stimulated murine macrophages (194). Moreover, 1,25(OH)₂D₃ treatment leads to the inhibition of the listericidal activity, the inhibition of phagocyte oxidase-mediated oxidative burst, and the suppression of IFN-γ-induced genes, including CCL5, CXCL10, CXCL9, FcgR1, FcgR3, and TLR2 (194).

However, many studies described the effects of 1,25(OH)₂D₃ distinct from its immunosuppressive capacity. Most importantly, 1,25(OH)₂D₃ or its analogs can initiate the differentiation of myeloid progenitors into macrophages (90, 266, 391). 1,25(OH)₂D₃ can also enhance the phagocytosis by monocytes (572). Administration of 1,25(OH)₂D₃ to mitogen-stimulated mononuclear cells caused an increase in prostaglandin E production (267). When added to peripheral blood monocytes, 1,25(OH)₂D₃ augmented IL-1 production (44). In addition, 1,25(OH)₂D₃ alters oxidative metabolism of monocyte/macrophage as demonstrated by human blood monocytes, which developed enhanced competence of secretion of H₂O₂ in the presence of 1,25(OH)₂D₃ (91).

Remarkably, 1,25(OH)₂D₃-treated macrophages exhibit enhanced antimicrobial activity (103, 304, 444), because 1,25(OH)₂D₃ regulates genes encoding antimicrobial proteins, such as the cathelicid and defensin B. The antibacterial effects of macrophages will be discussed in details in section VIII.F.

C. Effect of 1,25(OH)₂D₃ on DCs

A number of studies provided evidence that 1,25(OH)₂D₃ inhibits differentiation, maturation, and immunostimulatory capacity of human and mouse DCs (10, 43, 150, 176, 177, 405, 412). The effect of 1,25(OH)₂D₃ on inhibiting the maturation of DCs was dependent on VDR (176). Molecular characteristics of 1,25(OH)₂D₃-treated DCs include low surface expression of MHC-II and costimulatory molecules (CD40, CD80, CD86); upregulation of inhibitory molecules (ILT3) decreased production of IL-12 and enhanced secretion of CCL22 and IL-10 (43, 150, 176, 177, 405–408, 412). The inhibition of immunogenic pathways and the induction of “tolerogenic” molecules all together lead to a tolerogenic DC phenotype (352, 407). The tolerogenic properties of DCs induced by 1,25(OH)₂D₃ treatment are usually associated with the induction of Treg cells (FIGURE 5). However, tolerogenic pathways initiated by DCs can be also manifested in effector T-cell death and T-cell anergy. According to our study, 1,25(OH)₂D₃ can regulate transcription of several immune function-related genes, including genes the altered expression of which contributes to tolerogenic DC phenotype, independently of the differentiation process (507). The capacity of 1,25(OH)₂D₃-treated DCs to induce CD4⁺ CD25⁺ regulatory T cells that, besides its direct effects on T cells, can limit ongoing inflammatory responses make this ligand or its analogs (alone or in combination with other anti-inflammatory compounds) potential therapeutic targets (see below).

D. Endogenous Production of 1,25(OH)₂D₃ in Macrophages and DCs

1,25(OH)₂D₃ is ~1,000 times less abundant in human serum than its precursor, 25(OH)D₃ (206–208). This concentration is insufficient to turn on 1,25(OH)₂D₃-induced transcriptional changes in monocyte-derived DCs (507) and most likely in macrophages. Remarkably, a number of studies documented that both macrophages and DCs could produce 1,25(OH)₂D₃ from its precursors, suggesting that 1,25(OH)₂D₃ could act by an autocrine or paracrine fashion in these cells (8, 147, 171,
198, 269, 426–428). The key feature of endogenous 1,25(OH)2D3 production is the upregulation of enzymes involved in the synthesis of 1,25(OH)2D3. As we could previously see, the inactive 25(OH)D3 is converted to 1,25(OH)2D3 by CYP27B1 enzyme. (Most likely this second hydroxylation is the limiting step in antigen presenting cells.) Interestingly, while the expression of CYP27B1 in the kidney is inhibited by many signals [e.g., calcium, parathyroid hormone, phosphate, and 1,25(OH)2D3 itself (94, 143, 199, 364, 416, 429, 526)], antigen presenting cells seem to lack responsiveness to these inhibitory signals (7, 183, 199, 426). In contrast, CYP27B1 is upregulated in macrophages and/or DCs by various maturation/activation stimuli, including IFN-γ, IL-2, LPS, TLR2/1-activator, CL075, CD40 ligation, TNF-α, and poly(I:C) (6, 7, 147, 198, 304, 419, 426, 427, 507). In some cases, responses of macrophages and DCs are different. For example, synthetic 19-kDa Mycobacterium tuberculosis-derived lipopeptide upregulates CYP27B1 in monocytes and macrophages, but not in DCs (304). Some agents, e.g., LPS, TLR2/1L, and CL075, are able to induce the expression of both VDR and CYP27B1, leading to elevated receptor and ligand levels simultaneously. The inactivation of 1,25(OH)2D3 is carried out predominantly by the CYP24A1 enzyme, which is a direct VDR target gene.

E. The Potential Role of Endogenously Produced 1,25(OH)2D3 in Triggering Negative-Feedback Mechanisms

The fact that 1,25(OH)2D3 has immunosuppressive capacity suggests a potential negative-feedback mechanism in antigen-presenting cells (133, 182, 194, 198). According to this scenario, besides initiation of immunogenic responses, inflammatory stimuli can also induce the generation of a negative signal, 1,25(OH)2D3, that limits the extent of the immune response and helps to avoid over-stimulation (4, 95, 133, 194). As we could see in section VIII, B and C, endogenously produced 1,25(OH)2D3 can inhibit inflammatory pathways by various mechanisms. A recent study suggested another potential mechanism, demonstrating that 1,25(OH)2D3 can induce hyporesponsiveness to pathogens by downregulating the expression of TLR2 and TLR4 on monocytes (4, 451). The hypothesis of such a negative-feedback loop is supported by studies on VDR knockout animals. For example, VDR knockout mice are more resistant to intracellular protozoan Leishmania major infection than their wild-type littermates (133). Moreover, VDR knockout mice have larger subcutaneous lymph nodes than their wild-type littermates and contain increased number of mature DCs (176), most likely due to the lack of negative feedback of DC maturation.

F. Antimicrobial Role of 1,25(OH)2D3 in Macrophage

Detection of pathogens and initiation of immune responses are dominantly triggered by TLRs and other pattern recognition receptors (13, 226, 331, 415, 576). TLR2 activation by bacterial lipoproteins leads to killing of intracellular Mycobacterium tuberculosis in both mouse and human macrophages, albeit via distinct mechanisms (304, 517). In mouse macrophages, activation of TLR2/1 triggers the production of iNOS and consequently the release of nitric oxide (517). In human macrophages, the mechanism is nitric oxide-independent. The activation of TLR2/1 induces the expression of both VDR and CYP27B1, leading to an increased expression of VDR target genes, including antimicrobial peptides, cathelicidin, and defensin B2/4 (5) (167, 304, 548) (FIGURE 4). These peptides efficiently kill intracellular Mycobacterium tuberculosis. [TLR2/1-mediated expression of defb4 requires convergence of the IL-1β and VDR pathways (303).] Cathelicidin is not only necessary but also seems to be sufficient for vitamin D-mediated human antimicrobial activity (305). However, it is possible that this antimicrobial peptide is not the only mediator of antimicrobial activity (304). The identified 1,25(OH)2D3-dependent antimicrobial mechanism could explain a series of previous observations (302, 304, 305). First, both heliotherapy (sun exposure or ultraviolet light) and cod liver oil are efficient in the treatment of tuberculosis (reviewed in Ref. 422). A well-known acknowledgement of the success of such trials was Niels Ryberg Finsen’s Nobel Prize in Physiology or Medicine in 1903 for his contribution to the treatment of diseases, especially lupus vulgaris (cutaneous tuberculosis), with concentrated light radiation. Second, vitamin D deficiency is associated with tuberculosis (563), and VDR variations confer increased susceptibility to Mycobacterium tuberculosis infection in humans (38, 563). Third, 1,25(OH)2D3 induces an antimicrobial activity against Mycobacterium tuberculosis of cultured monocytes/macrophages (103, 442, 444, 476). Last, African Americans are known to have increased susceptibility to Mycobacterium tuberculosis infection (204, 304), most likely because of the lower level of vitamin D3 and cathelicidin D. Supplementation of the African American serum with 25(OH)D3 to a physiological range restored TLR induction of cathelicidin mRNA (204, 304).

Remarkably, correlation between insufficient vitamin D status and ulcerative colitis and Crohn’s disease was also documented (204). More recent findings illustrate how vitamin D deficiency may be a cause for these conditions. Nod2 (Card15) gene, encoding an intracellular pattern recognition receptor, has been identified as a direct target gene of VDR (547). Because Crohn’s disease pathogenesis is associated with attenuated NOD2 (218, 389), this study provides a molecular mechanism to explain the
connection between vitamin D deficiency and the genetics of Crohn’s disease. Another study demonstrated that impaired vitamin D leads to dysregulated colonic antimicrobial activity and impaired homeostasis of enteric bacteria (277). DNA array analysis of colon tissue also identified many genes, including angiogenin-4, an antimicrobial protein being consistently downregulated in vitamin D-deficient mice providing a pivotal mechanism linking vitamin D status with inflammatory bowel disease in humans (277).

G. Therapeutic Considerations of 1,25(OH)2D3

It has been estimated that 1 billion people worldwide have vitamin D deficiency or insufficiency [25(OH)D3 serum level below 50 nM] (204). Many aspects of vitamin D deficiency are discussed in details in a recent review (204). Rickets and osteomalacia are well known but are rather extreme examples of the effects of vitamin D deficiency. 1,25(OH)2D3 has a broad range of biological activities, and it is not surprising that vitamin D insufficiency can raise the risk for various diseases and susceptibility for infections. Living at higher latitudes (where UVB doses are lower) increases the risk of various types of cancer. Furthermore, correlations have been found between higher latitudes and risk of type 1 diabetes, multiple sclerosis, Crohn’s disease, hypertension, and cardiovascular disease (204). In parallel, vitamin D supplementation could reduce the risk for developing autoimmune diseases, e.g., multiple sclerosis, rheumatoid arthritis, and osteoarthritis, as well as of type I diabetes (204, 329, 337, 362). Although in many cases the correlation between D insufficiency and the risk for the disease is clearly demonstrated, the exact mechanisms for vitamin D effect and the target cell types are not defined.

Animal studies also demonstrated that vitamin D and its analogs can prevent the development of various autoimmune diseases, including systemic lupus erythematosus, experimental allergic encephalomyelitis, collagen-induced arthritis, Lyme arthritis, inflammatory bowel disease, and autoimmune diabetes in NOD mice (reviewed in Ref. 326). To some extent, they are also effective in the treatment of these diseases. In addition, VDR agonists prolong allograft survival in a variety of experimental models supporting a further potential pharmacological application for this hormone or its analogs (9, 326).

Vitamin D deficiency is also among risk factors of tuberculosis infection (381). Apparently, vaccination and antibiotics are the most effective way of prevention and treatment of tuberculosis, but vitamin D can also increase resistance to infection, especially in nonvaccinated people (385, 422). Clinical trials of adjunctive vitamin D therapy in active tuberculosis are reviewed in Reference 422.

IX. COMPLEXITY OF GENE REGULATION BY NUCLEAR RECEPTORS: FROM PATHWAYS TO NETWORKS

It appears that there are extensive interactions between nuclear receptor-regulated events and also other signaling and transcription networks. A nice documentation of such interaction between TLR and nuclear receptor signaling was provided by Glass and colleagues (388), who were able to show that while the glucocorticoid receptor-mediated transcriptional repression takes place by the disruption of the p65/IRF complex, PPARγ and LXR-mediated repression is independent of this pathway.

Here we attempt to classify the various modes of interactions and illustrate these with examples we already detailed above. Analyses of cell surface markers as well as functional tests and gene expression profiling demonstrated that agonists for RXR, RAR, LXR, PPAR, and VDR regulate overlapping sets of genes and functions in macrophages and DCs (508). This phenomenon is due to the fact that activation of nuclear receptors is resulted in parallel signaling and crosstalk between these receptors (FIGURE 7). These observations suggest that instead of isolated pathways, one must consider networks involving nuclear receptors, cofactors, and enzymes that participate in the production of active ligands. Others and also us could present evidence for this parallel signaling and cross-communication between nuclear receptors, many of them take place in macrophages and DCs.

Some examples are given here and are illustrated on FIGURE 7. We could identify four distinct modes of interactions. 1) Many natural ligands can activate more then one nuclear receptors, e.g., ATRA is a ligand for both RAR and PPARδ, while DHA can regulate both RXR and PPAR (116). RXR agonists induce several pathways in parallel, because they can regulate RXR homodimers and many permissive heterodimers, e.g., LXR:RXR and PPAR:RXR (508). The biological relevance of this redundancy is not clear. 2) One nuclear receptor can serve as a sensor and be activated by multiple ligands. For example, PPARγ is regulated by various modified fatty acids and prostanoids. 3) Some nuclear receptors are primary targets of other nuclear receptors, e.g., LXRα and PPARδ are regulated by PPARγ (77) and VDR (130), respectively. 4) One receptor can modify the activity of other nuclear receptors and transcription factors. This is carried out by regulating the expression of co-factors, enzymes, and scavenger receptors and by antagonizing other transcription factors. For example, RAR can induce the expression of RIP140, which could influence RAR and PPAR signaling (146, 558). A particularly intriguing example of the crosstalk is the regulation of endogenous ligand
production. There are multiple examples for such activity in macrophages and DCs. For example, we have shown that PPARγ can transform DCs to RA-producing cells providing ligand for RARs (504), also the dual regulation of CYP27 by RAR and PPARγ leads to increased ligand production for LXR (498). Nuclear receptors, e.g., GR, VDR, LXRs, and PPARs, are described and considered as negative regulators of many inflammatory responses. A unique crosstalk between these nuclear receptors and other transcription factors (e.g., NFκB, AP-1, and IRFs) has been documented in the process of transrepression (162), suggesting that one must investigate signaling pathways together to understand the effects of nuclear receptors. Novel technologies (e.g., microarray, ChIP-chip, GRO-Seq, RNA-Seq, additional new-generation sequencing technologies, etc.) revealed and continue to reveal another level of complexity of transcriptional networks on the promoter/enhancer regions of genes. These technologies are likely to be transformative in terms of providing large genome scale datasets. For example, C/EBPs were reported to bind to the vicinity of PPARγ response elements in adipocytes (282, 380). Recently, we could show such an interaction exists between PPARγ and the transcriptional mediator of IL-4 signaling STAT6 (497). This interaction and the facilitating role of STAT6 for PPARγ signaling provides a plausible explanation for the enhanced receptor activity in IL-4-exposed alternatively activated macrophages and DCs. Further similar interactions are expected to be uncovered by genome-wide localization and profiling approaches.

As more and more such interactions are revealed, the interpretation and analysis of data require the implementation of mathematical models. One can identify well-established regulatory logic circuit elements such as autoregulation of LXR expression in human cells (562), positive feedback loop in the induction of the scavenger receptor CD36 by PPARγ, which leads to increased oxLDL uptake and PPARγ activation. The level of endogenous ligands can be regulated by inducing enzymes involved in the ligand conversion. For example, PPARγ can transform DCs to retinoic acid producing cells providing ligand for RAR. One receptor could indirectly influence the activity of another nuclear receptor by regulating the expression of certain cofactors. For example, RAR can induce the expression of RIP140, which could influence RAR and PPAR signaling (NR1, NR2).

As more and more such interactions are revealed, the interpretation and analysis of data require the implementation of mathematical models. One can identify well-established regulatory logic circuit elements such as autoregulation of LXR expression in human cells (562), positive feedback loop in the induction of the scavenger receptor CD36 by PPARγ, which leads to increased oxLDL uptake and further activation of PPARγ or regulatory chains or cascades when RAR facilitates PPARγ signaling and that in turn activates LXR (77, 520) (FIGURE 7). Genome-wide analyses might contribute to the identification of further interactions and reveal the regulatory logic of lipid signaling via nuclear receptors in these cell types. These analyses eventually will lead to true system level understanding of these processes especially if integrated to other transcriptional networks such as NFκB, etc.

**X. LIGAND PRODUCTION BY DENDRITIC CELLS IS INVOLVED IN DETERMINATION OF T-CELL HOMING AND DEVELOPMENT**

Although it is a key issue to determine how endogenous ligands are produced for nuclear receptors in macrophages and DCs, it is also conceivable that these cells themselves may serve as sources of ligands for other cells of the immune system, therefore contributing to paracrine nuclear receptor ligand signaling.

**FIGURE 7.** Distinct logic circuits can be identified among the various interactions of nuclear receptor pathways in macrophages. A: “single input”: one ligand (L) can activate more than one RXR heterodimer (NR1, NR2, and NR3). For example, docosahexaenoic acid (DHA) can regulate both RXR and PPAR. B: “multiple input”: one nuclear receptor [NR] is activated by several agonists. For example, the relatively large ligand-binding pocket of PPARγ can bind a broad range of natural and synthetic ligands. C: nuclear receptors can regulate the transcription of their own (NR1) and other nuclear receptors (NR2-NR6). “Autoregulation”: LXRα regulates its own transcription in human cells. VDR regulates the expression of PPARδ. “Regulatory chain”: RAR enhances PPARγ response and PPARγ turns on LXRα gene expression. D: nuclear receptors can modify the activity of other nuclear receptors and transcription factors. Many nuclear receptors, e.g., VDR, LXRs, and PPARs, are considered as negative regulators of other transcription factors (TF) such as NFκB and AP-1. PPARγ regulates the expression of CD36 scavenger receptor (SR). Upregulation of CD36 leads to increased oxidized LDL uptake and PPARγ activation. The level of endogenous ligands can be regulated by inducing enzymes involved in the ligand conversion. For example, PPARγ can transform DCs to retinoic acid producing cells providing ligand for RAR. One receptor could indirectly influence the activity of another nuclear receptor by regulating the expression of certain cofactors. For example, RAR can induce the expression of RIP140, which could influence RAR and PPAR signaling (NR1, NR2).
A. Retinoic Acid Production by DCs

Emerging data suggested that various subsets of intestinal DCs can produce retinoic acid, more precisely ATRA, and this locally generated compound shapes the intestinal immune response via a paracrine modulation of lymphocyte development/activation. First, Iwata et al. (228) described that mesenteric lymph node (MLN) and Peyer patch (PP) DCs, isolated from mouse intestine, are able to produce ATRA in vitro (228). Consistent with this finding, these cells expressed several genes, which might be responsible for ATRA production, for example, MLN DCs expressed the transcript of alcohol dehydrogenase III and Raldh2 (aldh1a2) (228). ATRA is the active form of vitamin A, which is generated after two consecutive oxidation steps from retinol (vitamin A). First, retinol is oxidized to retinaldehyde (retinal) and then this compound is converted to ATRA. The first step is catalyzed by alcohol dehydrogenases and/or short-chain dehydrogenases/reductases (some of them are called retinol dehydrogenases), and the second irreversible step is catalyzed by retinaldehyde dehydrogenase (Raldhs) (129). It is still controversial which enzyme activities catalyze in vivo these reactions; however, genetic data indicated that, at least during early embryonic development, Raldh2 enzyme has a pivotal role (379). It seems that Raldh2 also had an important role for the production of DC-derived ATRA. The CD103+/subset of PP and MLN murine DCs highly expressed Raldh2, and these cells profoundly stimulated regulatory T-cell (Treg) development. Moreover, RAR antagonist (LE540, LE135) efficiently blocked Treg formation, suggesting that ATRA production was important for this step (97). Interestingly, a follow-up study revealed that a similar CD103+ DC population was detected in human mesenteric lymph nodes, and these human cells might also produce ATRA since the DC-dependent induction of gut homing receptors (CCR9 and α4β7) on T cells was blocked with a RAR antagonist (229). Gut-specific DC population is rather heterogeneous, a macrophage-like DC subtype (CD11c/CD11b double-positive cells) was isolated from the lamina propria, which expressed Raldh2 and produced ATRA (527). Of note, intestinal macrophages could also synthesize ATRA (121, 242); in addition, gut-associated stromal cells might also contribute to ATRA production (187, 343). These findings demonstrated that at least a few subsets of intestinal DCs can generate ATRA, and this is a gut-specific propensity of the DCs. However, a recent study described that a subset of migratory DCs derived from skin and lung was able to produce ATRA and induce Treg development, suggesting that ATRA-producing DCs are not restricted to the intestine (181).

It is intriguing which factors modulate the ATRA production capacity of DCs. It is possible that some antigen presenting cells (CD103+ DCs) have an inherent capacity to produce ATRA, more likely that the local environment instructs these cells to generate ATRA. Much effort has been applied over the past few years to identify the signaling pathways that modulate the ATRA production capacity of DCs. Our global gene expression profile analysis suggested that human PPARγ-instructed DCs generate ATRA. We found that several genes were upregulated, which participate in retinol/retinal oxidation (504). More importantly, our liquid chromatography-mass spectrometry analysis indicated that these PPARγ ligand-treated DCs produced ATRA ex vivo. There are conflicting reports on the effects of PPARγ activators in mouse DCs. Iwata and co-workers’ (578) analysis indicated that PPARγ ligand failed to induce RALDH2 expression in Flt3L-induced murine bone marrow-derived DCs; however, PPARγ activation enhanced Treg generation through increased ATRA synthesis in murine splenic DCs (215). It should be mentioned that our gene expression analysis revealed that besides the elevated expression of RALDH2, one of the retinol dehydrogenases (RDH10) was also upregulated in PPARγ ligand-treated DC. It is controversial whether the first or the second oxidation step is the rate limiting for the in vivo ATRA synthesis. Retinol conversion to retinal is catalyzed by many enzymes (at least in vitro), suggesting that this step has no role in the spatial or temporal regulation of ATRA synthesis (129). However, this notion has been challenged recently: RDH10 plays a critical role in embryonic ATRA synthesis, and loss of RDH10 function disrupts ATRA production during embryonic development (453). Consistent with this notion, it was also documented that a special gut-specific human antigen presenting cell (having shared DC and macrophage markers) also had an elevated expression of RDH10, suggesting that this enzyme activity might contribute to the ATRA generation at least in human cells (242). Our data also indicated that human monocyte-derived DCs had a relatively high expression of Ralldh2. Importantly, this in vitro DC differentiation is driven by IL-4 and GM-CSF cytokines; hence, it is possible that these cytokines might participate in the regulation of Ralldh2 expression. Indeed, IL-4 had a positive effect on the expression of Ralldh2 in mesenteric lymph node DCs, and IL-4 receptor-deficient cells had a reduced expression of Ralldh2 (134). Additionally, GM-CSF also stimulated Ralldh2 expression both in bone marrow-derived and splenic DCs, and this induction was enhanced in the presence of IL-4. Moreover, GM-CSF receptor-deficient animals showed an impaired intestinal ATRA production (578). Notably, in this report, SPF pathogen-free condition was applied since it turned out that TLR activators also enhanced RA production (578). In line with this finding, TLR2 ligand (zymosan) profoundly promoted the Ralldh2 expression in splenic DCs (320). In addition, this TLR2-dependent Ralldh2 induction was blocked with ERK kinase or syk kinase inhibitors, suggesting that these intracellular signaling path-
ways might participate in the positive regulation of this key enzyme of RA synthesis. Of note, intestinal DCs and macrophages derived from β-catenin null mice expressed much lower amounts of Raldh1 and -2 transcripts and proteins compared with wild-type cells. These data suggested that the β-catenin pathway directly or indirectly modulates the ATRA-producing capacity of the intestinal antigen-presenting cells (321). Interestingly, ATRA itself could directly induce Raldh2 in DCs, suggesting that ATRA enhances its own synthesis (344). Finally, a negative regulator of the ATRA synthesis was also identified: prostaglandin E₂ enhanced the expression of the inducible cAMP early repressor, which appears to directly inhibit RALDH expression in DCs (488). Taken together, these results demonstrated that at least in vitro several signaling pathways contribute to the regulation of DC-specific ATRA synthesis.

B. Modulation of Intestinal Immunity by DCs Via Production of Retinoic Acid

One of the major breakthroughs in DC biology was the deciphering of molecular mechanisms of gut-specific lymphocyte imprinting and oral tolerance (Figure 8). Several lines of evidence suggested that DC-derived ATRA signaling has a central role in these processes. It was previously observed that expression of the gut-homing receptors (α4β7 integrin and CCR9) was essential for the preferential homing of T cells to the intestine (185, 494). Iwata and co-workers discovered that ATRA enhanced the expression of these gut homing receptors (α4β7 and CCR9) in T lymphocytes. Moreover, they found that T cells that were primed in the presence of ATRA showed a preferential gut homing specificity (228). Notably, DC-derived ATRA was

**Figure 8.** The role of all-trans-retinoic acid and 1α,25-dihydroxyvitamin D₃ in T-cell homing and lymphocyte specification. A: schematic representation of the interaction between an activated DC and a lymphocyte. Nuclear receptor ligands are involved in the polarization and homing of lymphocytes. B: contribution of all-trans-retinoic acid (ATRA) and 1α,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] to the development of gut or epidermis homing phenotype of T cells, respectively. C: role and contribution of ATRA to the development of regulatory T cells and IgA-producing B cells.
also important for gut tropism in B cells (349). Numerous reports confirmed the ATRA-dependent induction of these receptors on T cells; however, it is still poorly characterized the ATRA-mediated regulation of αβ7 and CCR9. CCR9 expression seemed to be more dependent on ATRA than αβ7. Moreover, a functional cooperation was recently described between NFATc2 and the RAR:RXR heterodimer complex on the promoter region of the mouse Ccr9 gene, suggesting that ATRA directly modulates the expression of CCR9 (390).

In addition to T-cell tropism, DC-derived ATRA also facilitates the B-cell IgA secretion along with the commensal flora and TSLP. Moreover, vitamin A-deficient mice lacked IgA-secreting cells, suggesting the in vivo relevance of this regulation (349). After the identification of the gut homing tropism of retinoid signaling, it was found that ATRA also participated in the regulation of oral tolerance. Oral tolerance is the immunologic mechanism by which the gut immune system maintains unresponsiveness to antigens upon enteral uptake. Several lines of evidence suggested that inducible regulatory T cells (iTregs) have an important role in the maintenance of gut immune tolerance (105). Remarkably, intestinal DC-derived ATRA signaling elicited iTreg development via a synergy with TGF-β (355). This finding was confirmed by others, and an ATRA-producing DC subtype (CD103+ cells) was identified that modulates these processes (42, 97, 492). The mechanism of ATRA-dependent iTreg regulation is still poorly characterized; however, one report suggested that RARα activation directly induced histone acetylation in the promoter of Foxp3 (a master regulator of iTreg development) (105), hence activating this key gene (245). We have to note that it is still unclear whether ATRA directly stimulates iTreg differentiation or this is an indirect effect; one report suggested that ATRA indirectly regulates this process through the negative regulation of CD44 high T cells (201, 356) (FIGURE 8).

It seems that the effect of ATRA on T-cell regulation is more complex. Although it enhances TGF-β-mediated Foxp3+ T cell development, ATRA represses IL-10 production of these cells. Consistently vitamin A-deficient mice have enhanced IL-10-producing iTreg cells (328). Unexpectedly, ATRA was required to provoke a pro-inflammatory T-cell response to infection and mucosal vaccination. It seems that T cell specific RARα was the critical receptor of these effects: inhibition of the RAR signaling and deficiency in RARα resulted in a cell-autonomous CD4+ T cell activation defect (184). In addition, ATRA can elicit a proinflammatory phenotype in intestinal DCs to dietary antigens in a murine model of celiac disease. It was observed that ATRA synergizes with IL-15 to promote the breakdown of gluten tolerance and potentiate production of IL-12 and IL-23 by mucosal DCs (122). These unexpected results indicated that in a stressed intestinal environment, ATRA can act as an adjuvant that promotes rather than represses inflammatory responses. Taken together, these results suggested that DC-derived ATRA has a complex role on shaping lymphocyte activation and polarization in gut-associated lymphoid tissue.

C. Connection Between 1,25(OH)2D3 Released by DCs and Epidermal Lymphocyte Homing

Similarly to ATRA, 1,25(OH)2D3 was also implicated in ligand-induced lymphocyte trafficking (FIGURE 8). CCR10 is a chemokine receptor, expressed by 1% of CD8+ T cells and 5% of CD4+ T cells (474). Its ligand, CCL27, is a chemoattractant, selectively expressed by keratinocytes of the epidermis. This chemokine is thought to mediate and/or influence the epidermotropism of CCR10+ T cells from dermis, the retention of T cells in or near the epidermis, and the homing of cells from the blood into the vascular dermis (210, 351, 432, 474). The expression of CCR10 in human T cells and the migration of T cells toward CCL27 were significantly induced in the presence of 1,25(OH)2D3, especially when added together with IL-12 (474). Further experiments showed that naïve but not memory or previously activated T cells required IL-12 for efficient 1,25(OH)2D3-mediated induction of CCR10. In the promoter of CCR10, a DR3-type VDRE has been identified, suggesting a direct ligand-dependent regulation (474). 1,25(OH)2D3 alone or with IL-12 upregulate CCR10 in mouse T cells as well, albeit not as efficiently as in human T cells. The concentrations found in normal serum were ineffective to induce the CCR10 level of T cells. Importantly, monocyte-derived DCs themselves and T cell-DC cocultures could convert vitamin D3 to 1,25(OH)2D3, because both hydroxylation steps could be accomplished by DCs (see above). It should be noted that T cells could convert only 25(OH)D3 to 1,25(OH)2D3. These results do not exclude the possibility that other cell types, e.g., keratinocytes, could also process vitamin D to 1,25(OH)2D3 in the skin. In agreement with these results, 25(OH)D3 and vitamin D itself could induce CCR10 expression on T cells interacting with antigen presenting DCs. Sigmundsdottir et al. (474) could document the ability of skin-associated DCs to metabolize the sunlight-induced vitamin D using DCs obtained by cannulating an afferent lymph vessel draining site of chronic skin inflammation in sheep. The survey on the effect of 1,25(OH)2D3 on other molecules important for skin homing helps to address which aspect of T-cell migration is affected by 1,25(OH)2D3. Migration of effector and memory T cells relies on the expression of ligands for E- and P-selectin and CCR4 and CCR10 (348, 350). 1,25(OH)2D3 failed to induce the expression of CCR4 and other skin homing-associated chemokine receptors CCR6 and CCR8 (348, 350). Moreover, 1,25(OH)2D3 blocks the upregulation of ligands of E-selectin and the expression of fucosyl-
transf erase-VII, an enzyme essential for the synthesis of selectin ligands (316, 350, 474, 574). Remarkably, 1,25(OH)2D3 inhibited T-cell homing to the inflamed skin (574). These data collectively suggest that 1,25(OH)2D3 does not mediate T-cell recruitment from the blood. Instead, 1,25(OH)2D3 may act on T cells directly in the skin, after T cells infiltrated the dermis, inducing CCR10 to generate a T-cell attraction signal toward (and/or retention in) the epidermis.

XI. CONCLUSIONS AND PERSPECTIVES

The remarkable versatility and plasticity of macrophages and DCs makes them an ideal target for studying the activity of lipid-activated transcription factors, nuclear receptors. The last 15 years have brought a significant advance in this field. This was not in small part due to the availability of gene expression profiling and other genome-wide approaches. Now the contribution of retinoid, fatty acid, cholesterol, and prostanoid compounds via nuclear receptors on cell type specification and differentiation has been accepted by the immunology community.

From a molecular endocrinologist, “receptorologist” point of view, simply the large-scale identification of primary and secondary nuclear receptor targets proved to be very useful. At the same time, the relatively easy accessibility and availability of these cell types from both humans and mice makes these cells ideal for ex vivo culture and study. In particular, the possibility of analyzing multiple interacting pathways simultaneously in normal human or murine homogeneous cell populations is a unique opportunity. For example, issues such as the nature of RXR activation in the context of several heterodimers could be addressed. The identified target genes and pathways then could and should be verified and followed up in genetic models in vivo, which is a more tedious task, and these can contribute to a better understanding of receptor activity, interaction, and networking. Also, the molecular details of target gene activation and repression can be dissected at a genomic scale.

From an immunologist point of view, the introduction of nuclear receptors as modulators of immune cell differentiation and function provides an approachable system to probe the plasticity of macrophages and DCs and also to uncover the regulatory logic of immune cell differentiation, not in small part thanks to the availability of synthetic ligands. For example, we find it remarkable that simply applying lipids on DCs one can generate a range of activities leading to various T-cell subpopulations and also changing their relative amounts (FIGURE 5). These experiments also showed that activation of nuclear receptor pathways have specific effects rather than general peiotropic ones on the differentiation and function of immune cells.

A key outstanding issue is to determine to what degree these pathways are utilized in vivo and how endogenous ligand production is regulated. Also, it should be explored how these pathways interact with each other and with key inflammatory transcriptional responses such as NF-κB, AP1, or STATs.

From a more clinical and practical point of view, the identification and characterization of nuclear receptor-mediated pathways in these key immune cells are likely to contribute to a better understanding of disease mechanisms and potentially opening the door for the development for therapeutic approaches in chronic inflammatory diseases and autoimmunity. However, there is much more to do: more emphasis should be put on the systems level analyses, animal models in which genetic elimination of a particular receptor is possible in a given cell type at will; more thorough lipidomics to determine which are the active metabolic pathways and also more human clinical studies are required to avoid or bypass species specific phenomena and to convince even skeptics about the viability of such approaches and ease the translation of key findings to the clinic.

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