Dendritic cells and cytokines in human inflammatory and autoimmune diseases

Patrick Blanco [a,b,c,*], A. Karolina Palucka [d], Virginia Pascual [d], Jacques Banchereau [d]

[a] *CHU Bordeaux, 33076 Bordeaux, France*
[b] *CNRS-UMR 5164, 33076 Bordeaux, France*
[c] *Université Bordeaux 2, 33076 Bordeaux, France*
[d] *Baylor Institute for Immunology Research and Baylor Research Institute, Dallas, TX, USA*

Abstract

Dendritic cells (DCs) produce cytokines and are susceptible to cytokine-mediated activation. Thus, interaction of resting immature DCs with TLR ligands, for example nucleic acids, or with microbes leads to a cascade of pro-inflammatory cytokines and skewing of T cell responses. Conversely, several cytokines are able to trigger DC activation (maturation) via autocrine, for example TNF and plasmacytoid DCs, and paracrine, for example type I IFN and myeloid DCs, pathways. By controlling DC activation, cytokines regulate immune homeostasis and the balance between tolerance and immunity. The increased production and/or bioavailability of cytokines and associated alterations in DC homeostasis have been implicated in various human inflammatory and autoimmune diseases. Targeting these cytokines with biological agents as already is the case with TNF and IL-1 represents a success of immunology and the coming years will expand the range of cytokines as therapeutic targets in autoinflammatory and autoimmune pathology.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: Dendritic cells; IL-1; IL-12; IL-23; TNF-α; IFN-α

1. Introduction

The immune system is composed of a non-antigen-specific innate limb and an antigen-specific adaptive limb [1]. Innate immunity, borne by cells such as granulocytes and macrophages and proteins such as complement and cytokines, includes a variety of prompt reactions in response to infectious agents and other challenges. An excessive response results in inflammatory processes. The adaptive immunity, borne by lymphocytes, is acquired in weeks or months. It is characterized by an exquisite specificity for the eliciting antigen as well as memory, which allows a faster and stronger response upon re-exposure to the antigen. Adaptive responses can be immunogenic, leading to resistance to infections and possibly cancer, or tolerogenic avoiding response against self.

Indeed, to efficiently protect us from invading microorganisms, the adaptive immune system must distinguish self from non-self as immune responses against self can create a wide repertoire of autoimmune diseases. Anti-self immune responses are prevented through a variety of mechanisms occurring at various levels during the development of the immune system [2]. Autoreactive lymphocytes can be deleted, rendered anergic or rendered suppressive [3–5]. Suppressor T cells, also called regulatory T cells, suppress autoreactive responses both in an antigen-specific and a non-antigen-specific fashion. These immunological events happen either in the primary lymphoid organs (bone marrow and thymus) and are thus collectively called "central tolerance" or in the periphery and are then called "peripheral tolerance". Clinical autoimmunity arises as a result of an altered balance between the autoreactive cells and the regulatory mechanisms designed to counterbalance them.

DCs are specialized to capture and process antigens to present their peptides to lymphocytes. They are found in all

---

* Corresponding author. Tel.: +33 5 57 57 14 70; fax: +33 5 57 57 14 72.
E-mail addresses: patrick.blanco@chu-bordeaux.fr (P. Blanco),
Virginip@Baylorhealth.edu (V. Pascual).

tissues including blood and lymphoid organs [6–12]. In peripheral tissues, DCs are found in an immature stage specialized in the capture of antigens. In response to microbes, DCs undergo a complex process of maturation into antigen-presenting cells. This happens while the DCs migrate from the periphery into the draining lymph node through the lymphatics. In the steady state, DCs also migrate at a low rate without undergoing activation. Then they present self-antigens to lymphocytes in the absence of costimulation thereby leading to peripheral tolerance. Various mouse models have demonstrated that DCs bearing self-antigens are able to induce autoimmune diseases [13–15]. Furthermore alterations of DC homeostasis have been directly implicated in various human autoimmune diseases including type I diabetes, multiple sclerosis, and systemic lupus erythematosus (SLE) [16,17].

Here we review our current understanding of dendritic cell function in tolerance and how cytokines interfere with these processes to generate autoimmunity.

2. Dendritic cells

2.1. Dendritic cell maturation

Dendritic cells (DCs) are a heterogeneous family of cells of haematopoietic origin that are specialized in the handling of antigens, i.e. those from infectious agents and self, and their presentation to lymphocytes. Though most of the current knowledge relates to the presentation of peptides to T cells in the context of MHC classes I and II molecules, DCs can present glycolipids and glycopeptides to T cells and NKT cells as well as polypeptides to B cells. DCs undergo a complex maturation process from antigen-capturing cells into antigen-presenting cells. Numerous agents activate DCs including: microbes, dying cells, cells of the innate immune system and cells of the adaptive immune system. pathogen-associated molecular patterns (PAMPs) from microbes [18] signal DCs and other cell types through a variety of pattern-recognition receptors (PRR) including toll-like receptors (TLRs) [19,20]; cell surface C-type lectins receptors (CLRs) [21,22] and intracytoplasmic NOD-like receptors (NLRs) [23,24]. TLRs have been given the most attention until now and appear to be particularly important in the context of autoimmunity and most specifically SLE. Distinct DC subsets display different TLRs as will be discussed hereunder. Lysates of dying cells induce the maturation of DCs [25], and some components involved in dying cells enhance antigen presentation by DCs leading to T cell immunity [25,26]. These endogenous activating molecules are collectively called damage-associated molecular pattern molecules (DAMPs) [27]. They include heat shock proteins (HSPs) [28], high mobility group box 1 protein (HMGB1) [29], β-defensin [30] and uric acid [31].

DCs can secrete a diversified panel of chemokines that attract different cell types at different times of the immune response [32]. They also express a unique set of costimulatory molecules which permit the activation of naïve T cells and thus allow the launching of primary immune response. Through the cytokines they secrete, e.g.: IL-12, IL-23 or IL-10 as well as the surface molecules they express, e.g.: OX40-L [33] or ICOS-l [34] DCs can polarize naïve T cells into Th1, Th2, Treg or Th17.

2.2. Dendritic cell subsets

There are two main pathways of DC ontogeny from hematopoietic progenitor cells (HPCs). One pathway generates myeloid DCs (mDCs); while another generates plasmacytoid DCs (pDCs), a subset capable of secreting large amounts of type I IFN in response to viral stimulation [35,36] (Fig. 1). At least six DC subsets have been described in mouse spleen and lymph nodes, including conventional DCs (formerly designated myeloid DCs and lymphoid DCs) and plasmacytoid DCs [11,37]. They are distinguished according to surface markers such as CD11b, CD8a and CD11c, as well as by their functions [38–40]. Myeloid DCs are found in three compartments: (1) peripheral tissue, (2) secondary lymphoid organ and (3) blood. In the skin, two distinct types of mDCs are found in two distinct layers. Langerhans cells (LCs), which express CD1a and Langerin reside in the epidermis, while interstitial DCs (intDCs), which express DC-SIGN and CD14 reside in the dermis [41]. Plasmacytoid DCs, circulating in the blood and secondary lymphoid organs by crossing high endothelial venules, express BDCA2, ILT-7 and CD123, and secrete large amounts of type I interferons (IFNs) in response to viruses and/or TLR7–9 ligands [9]. Blood plasmacytoid DCs express TLR1, 6, 7, 9 and 10, but nor TLR4, while blood myeloid DCs express TLR1, 2, 3, 4, 5, 6, 7, 8 and 10, but not TLR9 [42,43]. Epidermal Langerhans cells isolated from skin lack the expression of TLR4 and TLR5, while dermal interstitial DCs express many TLRs including TLR2, 4 and 5 [44]. In the human, CLRs permit to distinguish DC subsets with BDCA2 specifically expressed on plasmacytoid DCs [45], Langerin expressed on Langerhans cells [46], and DC-SIGN expressed on interstitial DCs [47]. Many other C-type lectins are more promiscuous, and are, as is the case with TLRs, expressed on various cell types including endothelial cells and neutrophils. C-type lectins expressed on DCs act as anchors for a large number of microbes including viruses, bacteria, parasites and fungi, and allow their internalization, but they also act as adhesion molecules between DCs and other cell types including endothelial cells, T cells and neutrophils. Abnormalities in dendritic cell homeostasis have been implicated in various human diseases, including cancer, autoimmune diseases, allergy and infections.

2.3. Dendritic cells and immune tolerance

Central tolerance, induced in the thymus or bone marrow, plays a pivotal role in the prevention of undesired attacks
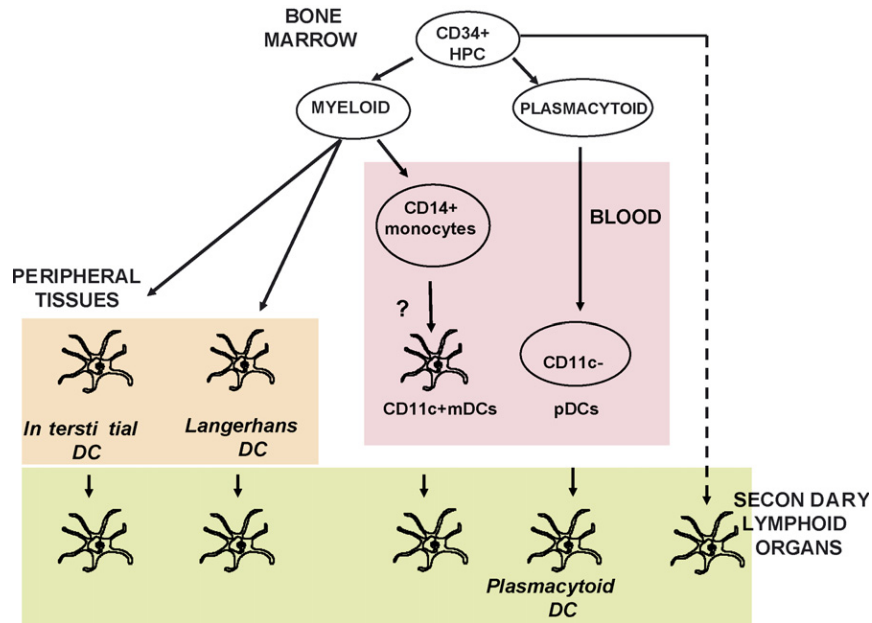
BONE MARROW

CD34+ HPC

MYELOID   PLASMACYTOID

CD14+ monocytes   BLOOD

PERIPHERAL TISSUES

?

CD11c-

CD11c+mDCs   pDCs

In tersti tial DC   Langerhans DC

SECON DARY LYMPHOID ORGANS

Plasmacytoid DC

Fig. 1. Dendritic cells are composed of subsets. DC progenitors originate from bone marrow CD34$^+$FLT3$^+$ hematopoietic progenitor cells (HPCs). A myeloid pathway generates both Langerhans cells (LCs), found in stratified epithelia such as the skin, and interstitial (int)DCs, found in all other tissues. It also generates mDCs circulating in the blood. Upon inflammation monocytes can yield mDCs. Another pathway generates plasmacytoid DCs (pDCs), which secrete large amounts of IFN-α/β after viral infection. Activated (mature) mDCs and pDCs traffic to secondary lymphoid organs either via afferent lymphatics (mDCs) or blood (pDCs). Langerhans DCs home to cell zones while interstitial DCs home to follicles consistent with their functional specialization, i.e. generation of cellular (Langerhans DCs) and humoral (interstitial DCs) immunity, respectively. The origin of resident lymph node DCs remains to be determined.

against self. Anti-self immune responses are prevented through a variety of mechanisms occurring at various levels of the immune system development [2]. Autoreactive lymphocytes can be either deleted, or rendered anergic or rendered suppressive [3–5]. Suppressor T cells also called regulatory T cells are also generated which suppress autoreactive responses both in an antigen-specific fashion and a non-antigen-specific fashion. Many peripheral auto-antigens through their expression in thymic medullary epithelial cells (a process regulated by the autoimmune regulator AIRE) are known to be responsible for the so-called negative selection [48]. Moreover, cytokines like thymic stromal lymphopoietin (TSLP) produced by epithelial cells of thymic Hassall's corpuscles promote the conversion of CD4$^+$CD25-thymocytes into CD4$^+$CD25$^+$Foxp3$^+$ T regulatory cells (Tregs) [49]. Dendritic cells in the thymus are also involved in the process of central tolerance [50].

It is clear, however, that negative selection in the thymus does not eliminate all autoreactive cells. Thus, tolerance induced in the periphery becomes a very important mechanism to maintain control of emerging autoreactivity. The mechanisms involved in peripheral tolerance are not entirely understood, but there is evidence that ''resting'' immature DCs that capture self-antigens in the steady state play an important role in this process. Indeed, under these conditions, DCs capture apoptotic bodies and/or cellular debris arising from normal cell turnover, migrate to draining lymph nodes and silence T cells reacting to these antigens [51]. The myeloid DC subset appears to be the most potent cell able to capture self-apoptotic bodies.

This phenomenon needs to be tightly regulated, as an unusual load of apoptotic bodies can induce systemic autoimmune disease [52]. Indeed, dead cells may also contribute to DC maturation, as it has been shown for necrotic or late apoptotic cells, but no early apoptotic cells. As discussed above, endogenous activating molecules are collectively called damage-associated molecular pattern molecules [27]. There is evidence that plasmacytoid DCs in their resting state are involved in tolerance induction [53–55]. pDCs stimulated via CD40 induce IL-10-secreting regulatory CD4$^+$ T cells [34] as well as suppressor CD8$^+$ T cells [56].

Recently, the concept that ''immature DCs are tolerogenic whereas mature DCs are immunogenic'' has been challenged by several studies showing that fully mature DCs can induce tolerance and differentiation of regulatory T cells [57–59]. In fact, the integration of different signals by the DCs, including Ag dose, cytokine milieu at sites of inflammation, encountered pathogen etc., will determine whether DCs will become tolerogenic vs. immunogenic. Possibly, peripheral tolerance is actively maintained by ''tolerogenic'' DCs [60]. In addition to deleting T cells, tolerogenic DCs induce the differentiation and proliferation of T cells with regulatory/suppressor functions [3,61]. Some pathogens have a capacity to actively render DCs tolerogenic [62]. Although the specific markers of tolerogenic DCs are yet to be determined, expression of inhibitory immunoglobulin like transcript (ILT) receptors might be their feature [63]. In vitro-generated DCs exposed to IL-10 express ILT-3, which is associated with their tolerogenic

functions [64]. Studies in mice suggest that DCs might be used in the treatment of autoimmunity through their ability to induce regulatory T cells. Thus, repetitive injections of "semi-mature" DCs induce antigen-specific protection of mice from experimental autoimmune encephalomyelitis and thyroiditis [57,58]. In NOD mice which spontaneously develop diabetes, DCs can induce the generation of Tregs *in vitro* which provide a therapeutic benefit even after onset of disease [59]. Indeed, Tregs appear to suppress DCs that induce autoimmunity by presenting autoantigens [59,65]. In keeping with this, animals which are depleted of Tregs show autoimmunity that is associated to expansion of activated DCs [66,67]. While immunogenic DCs have been used in clinical trials to treat mainly patients with cancer, understanding the mechanisms underlying the tolerogenic functions of DCs, such as those generated with IL-10 [68,69] or those infected with RSV [70], opens new avenues for the treatment of autoimmunity or the induction of specific tolerance in organ transplants.

Cytokines secreted to induce a specific immune response against an invading pathogen might interfere with DC homeostasis and induce an autoimmune response that can be responsible for tissue pathology. Indeed, clinical and epidemiological studies have suggested a link between infectious agents and chronic inflammatory disorders, including autoimmune diseases [71].

3. Cytokines, inflammation and autoimmunity

Cytokines represent critical mediators of the autoimmune process. They may represent products of DCs and/or induce the differentiation of immature DCs into mature DCs that can select autoreactive lymphocytes (Fig. 2).
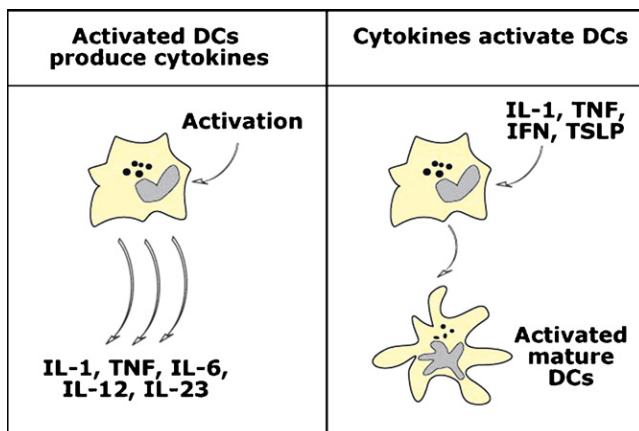

Fig. 2. Cytokines and dendritic cell activation. DCs both produce cytokines and are susceptible to cytokine-mediated activation. Thus, exposure to DC activators, for example TLR ligands or microbes, triggers secretion of pro-inflammatory cytokines including type I interferons (IFN), acute phase cytokines such as TNF and IL-6, IL-1 as well as IL-12 family (left panel). Several cytokines are able to trigger DCs activation (maturation) either in autocrine or paracrine fashion including IL-1, TNF, type I IFNs and TSLP (right panel).

3.1. IL-1 and its family

The IL-1 family plays an important role in inflammation and host defense. Up to 11 members of this family have been identified to date [72,73]. Of those, only five have been thoroughly studied: IL-1α, IL-1b, IL-18, IL-1RA and the recently reported IL-33. The remaining six (IL-1F5; IL-1F6; IL-1F7; IL-1F8; IL-1F9; IL-1F10) have been shown to be expressed in various cell types or tissues but their functions remain to be determined.

IL-1α and IL-1β are pro-inflammatory cytokines. Both are synthesized as precursor molecules (pro-IL-1α and pro-IL-1β) by many different cell types. Pro-IL-1α is biologically active and needs to be cleaved by calpain to generate the smaller mature protein. By contrast, pro-IL-1β is biologically inactive and requires enzymatic cleavage by caspase-1 in order to become active. IL-1α is primarily bound to the membrane whereas IL-1β is secreted and thus represents the predominant extracellular form of IL-1 (reviewed in Ref. [74,75]). IL-33 is a new member of the IL-1 family that is produced as a propeptide requiring cleavage by caspase-1. It binds to IL-1R4 (ST2) and stimulates T helper 2 (Th2) responses [73]. IL-1 is an activator of DCs, though it is not yet clear whether such IL-1-activated DCs display unique biological functions [73].

Interleukin-1 is involved in the pathogenesis of numerous diseases with an inflammatory component [76]. This is best demonstrated by the therapeutic benefits of treatment of patients with IL-1 antagonists such as IL-1-RA. These diseases include Systemic onset Juvenile Idiopathic Arthritis (SoJIA) [77], which represents up to 20% of chronic inflammatory arthritis in childhood. IL-1RA has also shown therapeutic efficacy in gout [78], type II diabetes [79] as well as a series of hereditary diseases causing periodic inflammatory symptoms and grouped under the term "familial autoinflammatory syndromes" [80]. Whether the beneficial effects are due to the inhibition of DC activation is not demonstrated.

3.2. IL-6 and its family

The IL-6 family is composed of IL-6, IL-11, leukaemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1) and cardiotrophin-like cytokine (CLC). Members of this family display pro- but also anti-inflammatory effects and play a central role in hemopoiesis as well as in innate and adaptive immune responses.

Activation of IL-6 signalling is mediated through the IL-6/sIL-6R complex, a process known as "trans-signalization" and a unique example of a soluble cytokine receptor displaying agonistic effects. IL-6 is secreted by many cell types, including B and T lymphocytes, monocytes, fibroblasts, keratinocytes, endothelial cells, mesenchymal cells and certain types of tumor cells. IL-6 induces the differentiation of B lymphocytes into plasma cells as well as

the proliferation of T lymphocytes, differentiation of cytotoxic T cells and IL-2 production. IL-6 also induces the differentiation of macrophages and megakaryocytes [81].

Recently, IL-6 has been described to participate in the differentiation of a novel T cell subset, Th17, which displays pro-inflammatory functions [82,83]. IL-23 is responsible for the expansion of Th17 previously differentiated, while IL-6 and TGF-β are responsible for the differentiation of Th17 from their naïve precursors. TGF-β induces Foxp3 which leads to the formation of Tregs, while IL-6 inhibits Foxp3 expression induced by TGF-β, and favors the formation of Th17 together with TGF-β [84–86].

IL-6 is likely to be involved in the pathogenesis of inflammatory and autoimmune diseases. It plays an important role in bone biology by inducing the differentiation and activation of osteoclasts and it mediates periarticular destruction of bone and cartilage in experimental models of arthritis [87]. IL-6 levels are increased in the serum of children with Systemic onset Juvenile Arthritis in a disease activity-dependent manner [88], and blocking its receptor is emerging as an effective therapy both in SoJIA [89] as well as in adult RA [90].

3.3. IL-12 and its family

IL-12, a heterodimeric cytokine produced mainly by activated myeloid DCs, plays a pivotal role in the differentiation and expansion of Th1 cells [91–95]. The recent discovery of IL-23 has led to a re-evaluation of interleukin-12 biology, as they share a common p40 subunit. In animal models, predisposition to autoimmunity can be explained by abnormal levels of IL-12 secreted by APCs [96]. Furthermore, IL-12 administration has been shown to switch tolerance mediated by intravenous or orally administered antigens into immunity [97]. In addition blocking IL-12 in patients with active Crohn's disease using a specific antibody can induce stable remission [98].

IL-23 is a cytokine that drives autoimmune diseases, including psoriasis and inflammatory bowel diseases [99,100]. IL-23 is secreted by human DCs exposed to gram-negative bacteria [101]. As mentioned above, IL-23 promotes the development and expansion of activated CD4$^+$ T cells that produce IL-17, IL-17F, IL-6 and TNF and are called Th17. Their differentiation is inhibited by IFN-gamma, IL-4 and IL-2 [102]. Genetic analysis of these Th17 cells identified a unique expression pattern of pro-inflammatory cytokines and revealed a unique role in different mouse models of autoimmune inflammation [103]. Given that the levels of IL-23 p19 and IL-17 are elevated in human diseases including multiple sclerosis, psoriasis and Crohn's disease, it is possible that these cytokines mediate human diseases [104–106]. Indeed, the therapeutic efficacy of an IL-12/23 p40 monoclonal antibody in psoriasis has recently been established [107]. The Th17 pathway has also been implicated in multiple sclerosis (MS) [108]. DCs, i.e. monocyte-derived DCs from MS patients, secrete more IL-

23 but equivalent amounts of IL-12 compared to healthy controls [104]. Patients with MS also appear to have increased numbers of IL-17-expressing cells [109]. Finally, a subset of infiltrating T cells express IL-17 in RA synovium [110].

The implication of IL-27 in autoimmunity is less clear as this cytokine can have pro- and anti-inflammatory properties [111,112].

3.4. TNF-α

TNF-α was among the first cytokines whose dysregulation was proposed to contribute to the pathogenesis of various autoimmune disorders. More importantly, TNF blockers have been extensively used and validated as an efficacious treatment for RA, Crohn's disease and psoriasis [113,114]. This clearly represents one of the greatest successes of immunology though the mechanisms of action remain unclear. Inasmuch as TNF induces many cell types, including DCs, to secrete pro-inflammatory cytokines, it is likely that TNF blocking results in their decreased secretion. Alternatively, anti-TNF-α therapy might generate a newly differentiated population of Treg cells distinct from natural Tregs, which seem to be defective in RA patients [115].

However, TNF antagonists are not without adverse effects, including reactivation of tuberculosis and induction of reversible systemic autoimmunity like SLE. In fact TNF blockers enhance the production of type I IFNs by pDCs exposed to viruses whereas TNF inhibits it. Type I IFNs, as described below, have been implicated as important mediators of autoimmune diseases in humans. Interestingly, transcription of type I IFN-inducible genes is observed in juvenile arthritis patients treated with TNF blockers. These data suggests that TNF represent an endogenous mechanism to control IFN production by pDCs [116]. Indeed, TNF produced by pDCs in response to viral activation acts as an autocrine maturation factor for these cells. Once they mature, pDCs are unable to secrete type I IFNs. It is therefore conceivable that blocking TNF would keep pDCs at an immature stage where they can continue to produce type I IFNs. Thus, based on these observations, immunity can be viewed as a dynamic system driven by opposite vectors, i.e. TNF-type I IFNs. The sum of the vectors yields an equilibrium point which allows protective immunity when vectors are equal. This dynamic system can accommodate the prevalence of either vector to a certain extent. However, when one of the vectors prevails beyond a certain threshold, the equilibrium point moves into a zone of immunopathology, including arthritis when the TNF vector prevails and SLE and others when type I IFN production prevails (Fig. 3) [16].

3.5. Type I interferons (IFNs)

Type I IFNs (IFN-α/β), major controllers of viral infections, play a role in several human autoimmune

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.

fastcase®
Smarter legal research.