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Immunological and Regulatory Functions of Uninfected and Virus Infected Immature and Mature Subtypes of Dendritic Cells—a Review

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Abstract. In 1868, dendritic cells (DCs) were discovered in human skin by Paul Langerhans using gold staining. These cells were named Langerhans cells (LCs) after their discoverer who, due to their dendrites, regarded them as neurons. One hundred and eleven years were to pass until it was discovered that in vertebrates these cells originate in the bone marrow as monocytes. In the 1980s, DC research was mostly carried out on DCs that are present in different tissues of mice and humans. These studies revealed that after interaction with foreign antigens, skin LCs/DCs migrate through the lymph vessels to the draining lymph nodes and induce the two arms of the immune response. The isolation of DCs from tissue cell suspensions opened the way to studies on the cells' surface proteins and their ability to stimulate immune responses. During the 1990s, studies revealed the role of DCs in the activation of naïve T cells in the lymph nodes and the regulatory properties of DCs in lymph nodes, thymus, gut, and spleen.

Part A of the review deals with the DC system of human and mice and immunological and regulatory functions of subsets of DCs in the skin with reference to migrating and stationary DCs, as well as the connection between DCs and the nervous system. Furthermore, the origin of both follicular DCs that are present in lymphoid tissues and thymic DCs are discussed. Part B is devoted to virus infections of DCs with an emphasis on infections caused by human herpes viruses. Part C presents the modulation of DC gene expression in response to the influenza virus. Contemporary research focuses on the role of DCs in the immune systems of vertebrates. Moreover, studies are being conducted on the regulatory functions of DCs by tissue cells in different organs of vertebrates.

Key words: dendritic cells (DCs), immature and mature dendritic cells, molecular biology of DCs, tissue specific dendritic cells, virus infections of DCs

Introduction

In 1868, Paul Langerhans, a medical student, used the gold staining method to study skin cell morphologies in human skin. As this was a new method with which to stain nerve tissues, Langerhans thus suggested that these dendritic cells (DCs) are nerves [1, 2]. His contribution subsequently led to the naming of these DCs as Langerhans cells (LCs).

One hundred years later, Breathnach et al. [3] reported that after transplantation of limb buds from 10-day-old mouse embryos that were deprived of their neural crest into spleens of histocompatible mice, LCs (but not Schwann cells or melanocytes) were observed in the developing mouse skin. Steinman and Cohn [4] identified DCs in peripheral lymphoid organs of the mouse and Katz et al. [5] reported that skin epidermal LCs derive from cells that originate in the bone marrow. These and other studies inspired active LC research during the 1980s that defined the intra-cellular structure and functions of these cells. The development of antibodies specific to LCs allowed for the identification and isolation of LCs from skin preparations in which LCs consisted

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of only 1–3% of the skin cell population. New technologies for identifying markers specific to LCs were developed, and it was soon reported that LCs are present in many mouse tissues—skin, thymus, lymph nodes, gastrointestinal tract, liver, lungs, heart, and genital tract. The immunological functions of LCs were reported by Roumani et al. [6]. A detailed review of the early studies on LCs was published [7] and a list of milestones in the field of DC research on DCs is presented in the next article [53].

Further studies on skin LCs revealed the involvement of DCs in humoral immune responses in vertebrates and their capacity to migrate from the skin to the peripheral lymph nodes in order to present antigens to T and B cells in the peripheral lymphnodes. Advanced molecular technologies provided novel information on gene expression of DCs during their existence in the skin, in an immature state, and during their transition to a mature state after interaction with foreign antigens, such as proteins, viruses, bacteria, and bacterial cell wall components. More recently, it was demonstrated that bone marrow-derived DCs can be differentiated into subsets of DCs whose functions are influenced by the epithelial cells in different organs of man, mouse, and chicken.

One of the aims of the present study is to review, in Part A, the available knowledge on epidermal DCs and the DC subsets of man, mouse, and chicken, and their immunological and regulatory functions will be discussed. In Part B, studies on the response of human and animal DC subsets to virus infection will be presented, with a special emphasis on herpes viruses (HSV-1, VZV, EBV, HHV-8 in humans) and MDV-1 in chickens. Part C illustrates studies that have been conducted on the modulation of DCs' gene expression during maturation and in response to infection by pathogens. Part D will discuss some of the riddles in the field of DC research that remain to be solved.

Part A: The DC System of Man and Mouse and their Immunological and Regulatory Functions

Skin LCs/DCs

Presentation of foreign antigens by LCs/DCs. The LCs/DCs are regarded as professional antigen-presenting cells that are present in the skin epithelium of man, mouse, chicken, and all vertebrates. They form a distinct layer of immature DCs throughout the skin, which is situated above the stratum granulosum layer of dividing keratinocytes. The bone marrow-derived precursors of DCs enter the blood vessels that carry them to all organs of the body. The DCs that enter the skin leave the blood vessels in the dermis of the skin and migrate to the skin epidermis.

Due to their relatively limited presence in the organs, methods were developed to induce blood monocytes to differentiate into immature DCs under *in vitro* conditions by incubating the DCs' precursor cells in cultures with granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin (IL)-4 [8]. The same method is used to expand the myeloid progeny of human $CD34^+$ bone marrow of precursors of DCs [9]. Such *in vitro* methods made possible studies on the molecular activities of DCs [10].

Macatonia et al. [11] reported that staining of the mouse skin with fluorescein isothiocyanate led to the concentration of the fluorescent compound only by the skin DCs and within 24 h the fluorescent DCs migrated from the skin epidermis to the draining lymph nodes. The migration patterns of DCs were reported by Austin and Larsen [12]. Studies revealed that DCs have the ability to interact with antigens ranging from low-molecular chemical compounds, peptides, particulate proteins, prions, viruses, and bacteria. The foreign antigens bind to cell surface receptors that are presented on the DCs' outer cell membrane and are consequently incorporated into the cell cytoplasm. The processed antigens are then transported to and presented in the antigen-binding grooves of HLA class I, HLA class II, and CD1 molecules. The interaction of DCs with antigens lead to the maturation of the DCs. These cells are triggered to detach from the skin keratinocytes and to migrate to the lymph vessels in which they transform into veiled cells that lack the typical dendrites. Upon arrival to the regional draining lymph nodes, the veiled cells develop dendrites that increase the surface of the DC cell membrane thus presenting multitudes of HLA class I, HLA class II, and CD1 molecules loaded with foreign antigens to naïve CD 8^+, cytotoxic T cells, and $CD4^+$ T helper cells.

It was reported that soluble proteins in pH sensitive liposomes are delivered to DCs, which induce cytotoxic T lymphocyte responses [13]. Incubation of bone marrow-generated DCs with HLA class I-restricted peptides made them potent inducers of cytotoxic T lymphocytes [14].

Maturation and trafficking of monocyte-derived DCs in a rhesus macaque model was investigated by Barratt-Boyes et al. [15]. It was reported that immature and CD40 ligand-mature monocyte-derived DCs have characteristic phenotypic and functional differences *in vitro*. Immature DCs express CC chemokine receptor 5 (CCR5) and migrate in response to macrophage inflammatory protein-1α (MIP-1α). Mature DCs switch expression to chemokine CCR7 and respond to MIP-3β. Immature DCs that traveled to the lymph node expressed CD86, which is indicative of spontaneous maturation.

The activities of skin DCs within the skin epidermis depend on the cytokines produced by skin keratinocytes that may influence their differentiation. Cytokines that include GM-CSF and TNF-α may be involved in the maintenance of the differentiation state of DCs/LCs. The contact between LCs and keratinocytes is mediated by E-cadherin, which contributes signals from keratincoyes to LCs. Since the skin epidermis contains type C nerve fibers, it stands to reason that neuropeptides that are released from the type C fibers into the skin epidermis may be involved in the regulation of the activities of the skin DCs/LCs [16]. This process will be discussed at length below. Treatment of the skin with chemical carcinogens, phorbol esters, steroids, IL-4, IL-10, and UV-B or X-ray is known to have inhibitory effects on the activities of LCs.

Skin LC subset that is intimately associated with the type C nerve fibers containing neuropeptide calcitonin gene related peptide (CGRP). Granstein et al. [16] investigated the type C fibers, axons of neurons that are present in ganglia and innervate the skin epidermis, that contain the neuropeptide CGRP in human skin. This research was carried out using specific antibodies to CGRP and to CD1a on DCs to identify the type C fibers and DCs/LCs, respectively. Double immunofluorescence staining revealed the network of DCs/LCs in the epidermis as well as the distribution of the CGRP positive nerve fibers in the skin epidermis. The authors indicated that most axons coursed the superficial dermis and epidermis in a serpiginous fashion and appeared with a beaded staining pattern of CGRP as single and branched processes, which were frequently found to have a Y-shaped bifurcation of the intraepidermal axons. Using confocal laser microscopy it was reported that 70–80% of the CD1 positive LCs were intimately associated with the CGRP positive type C fibers that terminated at LC bodies and not at cell dendrites. Occasionally, LC bodies showed patchy reactivity to CGRP antibodies due to the deposition of CGRP by nearby axons. This finding revealed that CGRP receptors are present in the outer membrane of DCs. The authors demonstrated that the addition of CGRP to isolated *in vitro* cultured LCs inhibited antigen presentation by the treated LCs. The authors concluded that these findings indicate that the release of CGRP by type C fibers in the skin may have immuno-regulatory effects *in vivo* as well.

Carucci et al. [17] reported that functional receptors for CGRP are present on human DCs and investigated potential immunomodulatory effects of CGRP on DCs other than LCs. The authors reported that treatment of immature DCs with CGRP (10^{-7} M) before and after maturation in monocyte-conditioned medium resulted in decreased cell surface expression of HLA class II DR and the constimulatory molecule CD86. Treatment with CGRP of mature DCs reduced the expression of CD86 but not HLA-DR. This study confirms the findings of Hosoi et al. [16].

A subpopulation of DCs expressing indoleamine 2, 3-dioxygenase (IDO). Munn et al. [18] reported on a subset of DCs that are present in lymphoid organs, which express indoleamine 2, 3-dioxygenase (IDO) that inhibits T cell proliferation through tryptophan degradation. The DC subset is identified by co-expression of the cell surface markers CD123 and the chemokine CCR6. The IDO positive DCs' suppressor activity was noted in mature and immature cells that can be found *in vivo* and may be regarded as the regulatory subset of antigen-presenting cells (APCs) in humans.

Follicular DCs (FDCs) in Lymphoid Tissues

FDCs are specialized DCs that display intact antigens for recognition by B cells in the spleen's germinal centers. MacKay and Browning [19] reported that the inhibition of lymphotoxin α/β (LT-α/β) production by B cells leads to the disappearance of multiple markers on FDCs in one day. Inhibition of the tumor necrosis factor (TNF) pathway was also inhibitory to FDCs. The authors indicated that these treatments prevented the capture of newly formed immune complexes on FDC cell membranes and eliminated previously trapped immune complexes on FDCs, thus

rendering these cells non-functional. Pretreatment of mice with inhibitors of the LT-α/β pathway before immunization with phycoerythrin similarly prevented the trapping of immune complexes on FDCs.

Matsumoto et al. [20] reported that in mice deficient in either LT-α or type I tumor necrosis factor receptor (TNFR-1) organized clusters of DCs and germinal centers were absent from the spleen of mice. The authors investigated the role of the LT-α and TNFR-1 in the establishment of spleen FDCs and germinal centers via reciprocal bone marrow transfer. When LT-α deficient mice were reconstituted with wild type bone marrow, FDC organization and the ability to form germinal centers were restored. This indicates that the LT-α expressing cells required to establish organized FDCs are derived from the bone marrow of the LT-α deficient recipient.

Recently, Marshall et al. [21] used a polymerase chain reaction (PCR) for cDNA subtraction to identify genes, which were specifically expressed in primary FDCs that were isolated from human tonsils. The authors reported on the discovery of a novel gene encoding a small secreted protein designated FDC-SP. The gene coding for this protein lies on the human chromosome 4q13, adjacent to clusters of proline-rich salivary peptides and C-X-C chemokines. Human and mouse FDC-SP proteins are structurally unique and the gene is expressed by activated FDCs from tonsils and TNF-α activated FDC-like cell lines, but are not expressed by B cell lines or primary germinal center B cells. FDC-SP peptides bind to the surface of B cells.

Thymic DCs are of Myeloid Origin

DCs are hematopoietic-derived APCs that display a potent ability to induce specific immune responses and delete potentially autoreactive T cells [22]. de Yébenes et al. [23] indicated that it appears as if the two non-overlapping functions of thymic cells result from the activities of two major DC sub-populations in the thymus, which have been characterized as myeloid and lymphoid DCs. In their study, [23] the authors reexamined the developmental origin of thymic DCs—a cell type considered to represent a homogenous lymphoid-derived population. The human postnatal thymocytes were isolated from thymus fragments, which were removed during cardiac surgery of patients aged 1–4 years (after attaining informed consent). The viable thymocytes were recovered and sequentially depleted of monocytes, as well as T, B, and NK cells by monoclonal antibodies-coupled magnetic beads. The authors proved that DCs are generated from early thymic progenitors and are truly of myeloid origins, as they stem from $CD34^{+}$ thymic progenitors, which have lost the capacity to generate T cells. The DCs' progenitors display myelomonocytic differentiation potential that is triggered by signaling through the GM-CSF/GM-CSFR pathway. GM-CSF is produced *in vivo* by thymic epithelial cells [23].

Using a different approach to identify the origin of thymic DCs, Radtke et al. [24] concluded that hematopoietic precursors, which lack the induced Notch 1 protein and are unable to generate T cells, retain all other myeloid and lymphoid lineages, including thymic DCs. The authors reported that Notch gene family members have been shown to have crucial roles in binary cell fate decisions in many developmental systems. This is due to the fact that Notch proteins are conserved transmembrane receptors, which contain EGF repeats in their ectodomain that are implicated in ligand binding. The cyotplasmic domain of the protein harbors six ankyrin repeats and is involved in intracellular signaling. Moreover, expression of transdominant active forms of Notch genes in transgenic mice uncovered the role of Notch signaling in T cell development at both the CD4 versus CD8 and α/β versus gamma/delta cell fate decisions. Notch signaling may also regulate the proliferation, survival, and apoptosis of developing T cells. Lethally irradiated mice recipients that were reconstituted with bone marrow from induced Notch $1^{-/-}$ mice were devoid of T cells in both the thymus and periphery. The authors proved that although Notch $1^{-/-}$ mice hematopoietic precursors are unable to generate T cells, they retained their full potential as far as the generation of all other myeloid and lymphoid linages are concerned.

Aiello et al. [25] reported that their analysis of a purified preparation of DCs revealed that they express high levels of inducible nitric oxide (NO) synthase (iNOS) and produced large amounts of NO. Additionally, peptides that are derived from MHC class II molecules upregulate iNOS expression and NO production by DCs. The authors suggested that DC-derived NO may be one of the soluble factors behind the regulation of events in the thymus that follow recognition of self and Allo-antigens.

DCs in the Chicken

Perez Torres et al. [26] reported that Ia positive and ATPase positive DCs are present in separated chicken epidermal sheets. These cells resemble skin LCs that are present in human and mouse skin epidermis. Gallego et al. [27] identified cells that react with a monoclonal antibody, which identifies FDCs that are present in the germinal centers in the chicken spleen. In a later study, Gallego et al. [28] observed that the FDCs, which were identified after intravenous priming with bovine serum albumin (BSA) and boosting with biotinylated BSA that conjugated to colloidal gold particles, had an irregular morphology that varied with time. Consequently, when FDCs in the chicken spleen showed filiformic cell dendrites, antigens were bound to their cell membrane.

Gallego et al. [29] also reported on chicken ellipsoid-associated cells, which are considered to be splenic DCs that migrate from the spleen into the blood after binding antigen to their cell membrane. The authors traced the localization of these cells within two peripheral lymphoid organs: the cecal tonsils and the Peyer's patches. The antigen binding cells were found in the diffuse lymphoid tissues and in the germinal centers in both lymphoid organs. These studies demonstrated that chickens have a DC system that is similar to other vertebrates, but detailed information on these cells is still limited.

Part B: DCs and Virus Infections

DCs serve as the immune system sentinels against pathogenic agents and foreign chemical substances that are disseminated in human and vertebrate skin epidermis. They carry out this role by forming a layer above the stratum granulosum in the skin epidermis that covers the entire body. The research on virus infections in humans and animals revealed that the infected host responds to virus infection by induction of the two arms of the immune system: the early immune response is the appearance of antiviral cytotoxic T cells ($CD8^+$ and $CD4^+$) and the late response is the synthesis of antiviral antibodies. In 1976, Nagao et al. [30] were the first to observe that after the inoculation of vaccinia virus to the human skin, virus particles were found in the skin LCs at the site of the virus administration. Recent developments in the research on virus infections of human and animal DCs will be discussed below.

The Role of DCs in Infections that are Caused by Herpes Viruses

Human herpes virus-1 (herpes simplex viruses 1 and 2 (HSV-1 and HSV-2))

(i) The ways whereby HSV escapes from the host's immune system. HSV-1 and HSV-2 are human pathogens that use the damaged human skin epidermis as a port of entry into the human host's skin, mouth, and genitals to infect the skin epithelial cells. During virus infection of epithelia, the virus progeny infects the type C fibers' axons of neurons in peripheral ganglia that are present in the epidermis, which transfer the virus to the neurons in the trigeminal or sacral ganglia. In the neurons the viral DNA achieves a latent state until the neurons are triggered to allow for virus replication. The nature of the pathway by which the virus migrates from the skin to the neurons in the peripheral ganglia to establish a latent infection and the mechanisms that trigger the virus reactivation in the neurons is not fully understood. The transfer of viral DNA by the neuronal axons to the skin epidermis needs to be defined. HSV resides in the infected ganglia of the human host throughout its life, and the latent virus may remain dormant for long periods or reactivates numerous times. The mechanism of the induction of latent HSV-1 is not understood.

HSV-1 is also capable of infecting the nasal and pharynx epithelia and the neuronal axons present in the epithelial layer transport. The virus DNA migrates by the axonal transport mechanism to infect the olfactory bulbs in the nose and the trigeminal ganglia, respectively [31]. Since DCs are present in the skin, nose, and pharynx epithelia to protect against invading viruses it is necessary to review the nature of the interaction between epidermal DCs and HSV-1.

(ii) HSV-1 infects immature and mature DCs. Mikloska et al. [32] studied the HSV-1 infection of immature monocyte-derived DCs (MDDC). CD14 positive cells were selected from peripheral blood and immature DCs were generated under *in vitro* conditions during 6–7 days of incubation in complete medium supplemented with 200 u of GM-CSF and 250 u of IL-4/ml. Following this period in culture, 95% of the cells differentiated

into CD1a and CD11 positive cells. These immature DCs were infected with a clinical isolate of HSV-1 at different multiplicities of infection. The infected cells were washed to remove unadsorbed virus and were further incubated in a medium containing GM-CSF and IL-4. The authors reported that HSV-1 infection of the immature DCs lead to a productive virus infection in 80% of the DCs that produce and release infectious virus progeny. The nature of the 20% DCs that were found to be virus resistant was not defined. The authors indicated that the immature DCs had surface characteristics that were more similar to skin LCs than to blood DCs and suggested that the *in vitro* differentiated DCs resemble in their sensitivity to HSV-1 to the natural HSV-1 infection in human skin *in vivo* where LCs/DCs are attached to the skin keratinocytes and are most probably influenced by the cytokines that are released from the keratinocytes.

The finding of Mikloska et al. [32] also revealed that HSV-1 infection of immature DCs leads to a marked decrease in the key functional viral surface molecules CD40, CD54, CD80, and CD86 within the first 24 h after infection. HSV-1 infection of the immature DCs did not affect the cell surface molecules: CD11c and HLA classes I and II molecules.

Sailo et al. [33] used a HSV-1 recombinant capable of a single replicative cycle in immature DCs and reported that the infected DCs could not produce cytokines and co-stimulatory proteins. The virus infected DCs also failed to acquire responsiveness to the CC-chemokine-7 receptor (CCR7) ligand, which appears to indicate an inability to migrate to lymph nodes. Nevertheless, the virus-infected DCs were capable of producing type I interferon and induced Th1 polarization. The infected DCs upregulated the synthesis of MHC class II and CD1a molecules. The authors viewed their findings as suggesting that the ability of HSV-1 to infect immature DCs and prevent their maturation could be utilized as an effective virus strategy to evade the antiviral immune response.

Kruse et al. [34] generated mature DCs from non-proliferating blood progenitors and reported that 90% of the cells were infected by HSV-1. The authors reported that CD83, a member of the immunoglobulin super family, a marker for DCs' maturation, is down regulated in HSV-1 infected DCs and the allogeneic T-cell stimulatory capacity of the virus infected DCs is affected.

The studies on the HSV-1 infection of immature and mature DCs that were derived from blood monocytes under *in vitro* conditions, may be taken to indicate that DCs have virus specific receptors on their cell membrane that bind the HSV-1 virus [35]. Furthermore, the uncoated viral DNA is capable to express virus genes α, β, and γ [32] and virus progeny is produced. HSV-1 utilizes the DCs cellular transcription factors to transcribe immediate-early virus genes. The immediate-early viral proteins are capable of interfering with critical cellular functions of the infected DCs to signal to the immune system cells that a HSV-1 infection has started. HSV-1 inhibits the transport of antigenic viral peptides by transporter proteins TAP 1 and TAP 2 to HLA class I molecules in the membrane of endoplasmic reticulum (ER) by the viral protein (ICP47) that is coded by the viral gene Us12. The viral protein ICP27 inhibits the splicing of cellular mRNA in the splicesome by a protein coded by the viral gene UL54.

In the human host, shortly after HSV-1 infection in the skin epidermis, it appears that immature DCs adsorb HSV-1 virions that bind to DEC-205 multi-lectin endocytosis receptors. The DEC-205 receptors are a trans-membrane protein containing 1,722 amino acids (aa) with externally disposed C-type lectin domains [36]. If indeed HSV-1 virions bind to the endocytosis receptors that are present on the DCs in the skin epidermis, the virions will be incorporated into endocytic vesicles, in which the viral DNA will be uncoated and transported to the cell nucleus. The viral structural proteins after degradation by the proteasomes will be transferred as viral antigenic peptides and presented on HLA class I or II molecules by a different mechanism. The HLA class I or II will be transported to the surface of maturing DCs. The mature DCs will migrate to the draining lymph node by the lymph channels as veiled cells, where they will resume their dendrites. This will allow naïve T cells to interact with the HLA molecules and induce the cellular and humoral immune responses that occur in HSV-1 infected people. In addition, epidermal DCs may phagocitise fragments of lysed virus infected keratinocytes and their cell membranes that contain the viral glycoproteins by binding to the endocytosis receptors. The mature DCs process the viral proteins and migrate to the lymph nodes to present viral antigens to T cells. HSV-1 may bind to specific receptors on the DC cell membranes using the viral glycoproteins.

(iii) Herpes simplex viruses use the DC host skin defense mechanism to migrate to neurons in order to establish a reactivable virus latency in the nervous system. The human skin epidermis and nasopharyngial epidermis that serve as the ports of entry for HSV-1 harbors epithelial cells, DCs, dendritic T cells with γ/δ receptors, and Merkel cells. These epidermal cells are in contact with the epithelial cell layers. Since the skin is the body organ that faces the outside world, it also serves as the sensing organ for temperature changes and signals with a pain signal to deleterious changes in the skin. Indeed, the study by Hosoi et al. [16] revealed that the type C non-myelinated fibers, which contain the neuropeptide calcitonin gene related peptide (CGRP) are axons of neurons that are localized in peripheral ganglia. The axons transverse the epidermal basement membrane, enter into the skin epidermis, and in specific regions of the skin they contact the cell bodies of the resident DCs. The type C nerve fibers are non-myelinated and function as transmitters of heart and pain signals to the pain center in the brain by responding with the release of CGRP into the epidermis. It was also reported [16] that the skin DCs are the only cells that harbor CGRP receptors. The damage caused by virus infection of the skin keratinocytes causes the local release of CGRP from the type C fibers and emits a pain signal to the pain center in the brain. Becker [37] suggested that the transport of HSV-1 DNA to the neurons in the ganglia starts with infection of the DCs that are connected with the type C fibers by Hosoi et al. [16]. The viral DNA–protein complexes may be transported directly to the type C fibers that will transport the viral DNA by the retrograde neuronal transportation mechanism to the neurons in the ganglion. The latency of the viral DNA molecules that enter the neurons' nuclei starts with the transcription of the latency (LAT) gene that codes for the latency RNA. This viral RNA, which is not translatable, prevents the controlled neuronal cell death (apoptosis).

Reactivation of the latent HSV-1 DNA is thought to be initiated in latently infected people when a sharp pain is felt at the site of the primary HSV-1 infection in the skin before virus infection erupts. The pain signal may be caused by the release of CGRP from the type C fibers in the epidermis due to unknown triggers. It was hypothesized [37] that the reactivation of the latent HSV-1 DNA in the neurons parallels the signal to the neuronal cell to replenish the CGRP peptides that were released in the skin. The neuron's transcription mechanism is reactivated and the transcription factor NFKB is reactivated by a two-stage mechanism and transported to the nucleus to transcribe the CGRP gene. At the same time, immediate-early genes in the viral DNA and subsequently the β genes may be transcribed, leading to virus DNA replication. Vesicles that contain the neuropeptide CGRP are transported by the type C fibers to the skin by the aenterograde transport mechanism of the axons, transporting also the HSV-1 DNA by the same mechanism. This idea is in agreement with Penfold et al. [38], who reported that viral DNA–protein complexes are transported from the neuron to the distal regions of the axons. It was suggested [37] that HSV-1 DNA is transported to the DCs that are connected with the type C axons [16]. The DNA–protein complexes are transported into the DC cytoplasm and the viral DNA may enter the cell nucleus to initiate infections virus progeny. Since Penfold [38] showed that viral DNA–protein complexes are transported in nerve fibers (axons) release of such complexes in the skin will not be able to infect keratinocytes. The virus progeny from DCs will be capable of infecting the keratinocytes. Additional studies are needed to determine the validity of the hypothesis

Infection of DCs by human herpes viruses (HHV)

(i) Varicella-Zoster virus (VZV, HHV-3) infects immature DCs. Anderoth et al. [39] reported that VZV infects and replicates in human monocyte-derived DCs and the infected DCs are capable of transferring the virus to T cells and fibroblasts *in vitro*. VZV-infected DCs maintain cell viability and the cell surface levels of HLA classes I and II, CD86, CD40, and CD1a are unchanged.

(ii) Human cytomegalovirus (HCMV, HHV-5) infects and replicates in DCs. Riegler et al. [40] reported that HCMV strains that are propagated in endothelial cell cultures infect immature dendritic cells that are generated from peripheral blood monocytes at a level of 80–90% of the cells. The virus replicated in the DCs and produced a virus progeny. However, fibroblast-adapted HCMV strains failed to cause significant cytopathogenicity in immature DCs.

(iii) HHV-6 infects DC. Asada et al. [41] reported that HHV-6 infects and replicates in immature DCs

that derive from blood mononuclear cells. Coinfection of the immature DCs with HIV-1 and HHV-6 inhibits HIV-1 replication in coinfected cells.

(iv) Epstein–Barr virus (EBV, HHV-4) infects FDCs. Lindhout et al. [42] reported that freshly isolated FDCs express the EBV receptor CD21 and that the infection of these cells with EBV resulted in the development of EBV transformed cell-lines that express EBV nuclear antigen 2. Shek et al. [43] reported on a patient with a primary follicular cell tumor of the liver. *In situ* hybridization for EBV RNA was positive in tumor cells in the original and recurrent tumors. The tumor cells showed identical episomal clonal EBV DNA, and expressed the Latent Membrane Protein.

Van Gorp et al. [44] studied 50 patients with Hodgkin's disease for the presence of EBV RNA using *in situ* hybridization and reported that a strong correlation existed between the presence of EBV RNA and Latent Membrane Protein 1 expression in Hodgkins cells (Read–Sternberg cells).

(v) Marek's disease herpes viruses infect DCs in the chicken. Davidson et al. [45] studied the presence of DCs in touch preparations of embryo tissues that were infected with oncongenic MDV-1 or MDV vaccine viruses. DCs were found mainly in the thymus and spleen of normal embryos. Immunoglobin positive cells, presumably FDCs, were found in the bursa, thymus, and spleen. Infection of embryos with pathogenic MDV-1 and vaccine viruses altered the distribution pattern of the DCs in a serotype specific manner: to a lesser extent, infection with MDV-B and SB1-induced the appearance of DCs in the chorioallantoic membrane, while HVT and CVI988 viruses depleted the DC populations from all organs except the bursa and the thymus.

Bobryshev et al. [46] reported that cells with atypical appearance of DCs were found to be present in the hearts' aortic lesions of Marek's disease infected chicken.

Part C: Modulation of DC Gene Expression in Response to Influenza Virus

Cella et al. [47] reported that human immature DCs that were generated *in vitro* from peripheral blood monocytes mature after the influenza virus infection. The maturation results in the production of type I interferons (INFs) and expression of the protein MxA [48], which protects DCs from the lethal effect of the virus. Pavlovic et al. [48] reported that human and mouse Mx proteins inhibit different steps of the influenza virus multiplication cycle.

Huang et al. [49] used immature human DCs that were differentiated from elutriated human monocytes after incubation for 7 days in standard medium, which contain GM-CSF and IL-4. The immature DCs were exposed to different pathogens (*E. coli*, influenza virus or *Candida albicans*) and the reprogramming of DC gene expression during the maturation process was studied using oligonucleotide microarrays. In the present review, attention will be given to the response of DCs to infection with influenza virus, since a similar study on HSV infected DCs has yet to be reported. The possibility exists that the response of immature DCs to HSV-1 infection may resemble the reprogramming of gene expression in influenza virus-infected DCs.

The authors reported that the same 166 activated cellular genes were the basic response of the infected DCs to each of the three pathogens. Additionally, influenza virus infection of DCs led to the expression of additional 58 specific cellular genes, but the innate immune response to the virus was devoid of the expression of genes capable of stimulating neutrophils. The interferon α and β genes (INF-α and INF-β) were markedly induced. A subset of genes that were induced in DCs by the influenza virus infection are linked to the inhibition of the immune response. These genes include pro-apoptotic genes that may lead to the early death of infected cells, genes that encode mcp-1, which is capable of blocking IL-12 production in macrophages, Gfrp, a gene coding of a protein that inhibits NO synthesis, and the gene that codes for the IDO, which inhibits T cell activation [49]. Furthermore, genes that are involved in diverse cellular functions also were modulated in the influenza infected DCs.

Huang et al. [49] reported that influenza virus induce several interferon genes in the infected DCs as well as the genes Mx1 and Mx2 that are expressed transiently. It is of interest that Pavlovic et al. [48] reported that the expression of human Mx genes in transgenic mice enhanced resistance to influenza virus infection [48].

Part D: Conclusions

The study of DCs in human skin that was initiated by Paul Langerhans in 1868, who suggested their neural properties, was delayed for an entire century. Only during the 1960s was it demonstrated that DCs are not of neural crest origin. During the 1970s, a significant breakthrough was achieved with the discovery that DC precursors are produced in the bone marrow of vertebrates. The initial phase was the development of specific monoclonal antibodies and staining procedures to identify DCs in animal and human tissues. These studies allowed for the tracing of DC migration from the skin epidermis to the draining lymph nodes. Thereafter, research on the involvement of DCs in the immunological responses of uninfected and virus-infected mice was initiated. Studies during the 1980s demonstrated that DCs are the professional APCs in vertebrates. Moreover, in their absence, due to physical or chemical treatments of the skin, the immune system was unable to protect the host against a virus infection. A detailed account of the milestones in LC/DC research, from their discovery in 1868 by Paul Langerhans until 1989, appears in the following paper [50]. The research on DCs during most of the 1980s was done with relatively limited numbers of DCs that were isolated from animal or human tissues. In the early 1990s, studies on the involvement of DCs in the immune responses of animals and humans led to the development of technologies that allowed for the production of a large number of DCs by differentiation of monocytes to DCs under *in vitro* culture conditions. This technology facilitated detailed immunological studies on DCs. Most notable among those innovations was the development of DC elutriation of human blood monocytes and their differentiated into immature DCs after incubation in a medium containing GM-CSF and IL-4. These studies led to rapid advancements in the understanding of DC functions as well as studies on the molecular biology and gene expression of DCs.

Although immunological studies using *in vitro* differentiated DCs lead to important findings, there is a need to study DC maturation under *in vivo* conditions. Immature DCs reside in different organs of humans and animals. In each organ or tissue, they are exposed to different cytokines and chemokines that are produced by the specific tissue cells in which the DCs reside. For example, the DCs precursors that are produced in the bone marrow and transferred by the blood to all the tissues of the animal are not fully differentiated and remain immature DCs even when they reside in the tissue epithelium. In the blood, the progenitors of DCs most probably encounter the neuropeptide CGRP, which was shown to inhibit the DCs immune function [16]. Another example of this is the discovery that FDCs require lymphotoxin α and β to mature, which is provided to them by B cells in the germinal centers of the lymph nodes. Thus, it seems necessary to study DCs in different organs under *in vivo* conditions in order to define the cytokines and chemokines that determine the differentiation and maturation of DCs in specific organs.

Another unresolved property of DCs is their capability to regulate different cell types. The studies of Munn et al. [18] and Hwu et al. [51] identified a subset of DCs that are IDO positive and are capable of inhibiting T cell proliferation. It is of interest that Huang et al. [49] reported that the expression of IDO gene was stimulated in DCs by infection with influenza virus, which suggests that the influenza virus infected DCs are capable of inhibiting T cell proliferation. Moreover, the positioning of DCs in human skin as a layer of cells in the epidermis above the stratum granulosum—the epidermal site of keratinocyte proliferation—is another example that DCs may function to regulate keratinocytes proliferation in the skin epidermis. It is common knowledge that exposure of the human skin to sun rays leads to skin damage that is followed by the peeling off of keratinized layers of skin. This is most probably due to the enhanced division of keratinocytes. Enk [52] reported that irradiation of human keratinocytes *in vitro* with UV-B induces the production of IL-10 by the keratinocyts. IL-10 is an inhibitor of DCs. It may be assumed that in skin that has been exposed to the sun, the IL-10 produced by the irradiated human skin inhibits the DCs regulatory activity. Further studies on the responses of DCs to physical and chemical agents and to different cytokines and chemokines using gene expression technology [49] may provide information on the modulation of immunological and regulatory functions of DCs by different tissues cells *in vivo*.

The studies on the susceptibility of *in vitro* generated DCs to viruses need to be refined as far as defining virus receptors under *in vitro* and *in vivo* conditions to determine the ability of these cells to travel to the lymph nodes after virus infection. It is

not yet known how influenza virus infections in humans affects the lung DCs, the host defense mechanism that protect the lungs from pathogens and the involvement of DCs in the protection against retroviruses and prions, to name a few. The development of synthetic vaccines to protect against virus infections also depends on the knowledge of the DCs and their ability to introduce foreign antigens (e.g. synthetic peptides) into the peptide-binding grooves of HLA class I molecules while in the human skin. An approach to the development of epidermal synthetic peptide vaccine for skin application was reported [53].

Many questions on the functional and immunological properties of DCs were answered over the last 40 years, but DCs still maintain secrets that must be deciphered.

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