

MINERVA

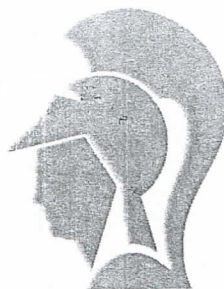
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infection. Tissue injury and inflammation can cause dramatic changes, including release of mediators such as cytokines and chemokines, that shape protective immune responses. Monocyte-derived mature DC are currently generated *in vitro* by 2 sequential steps, leading first to the immature DC (iDC), and then to mature DC (mDC), after the addition of different stimuli. However, there are no reports on the involvement of chemokines in the modulation of DC maturation. Chemokines are a large family of proteins initially interpreted as leukocyte chemoattractants, but since shown to modulate a larger array of biological functions. We demonstrated for the first time that CCL16 may be an important costimulator of cytotoxic functions crucial for augmenting local target cell destruction and its presentation to T cells achieving a new biological role in orchestrating an antitumor immune response¹. The aim of this study was to investigate CCL16's effects on human DCs. We evaluated the morphology, the downstream production of chemokines and cytokines, the T cell stimulatory capacity in an allogeneic MLR and the antigen (Ag) uptake ability of DC matured in the presence of CCL16 alone (mDC/CCL16) or a mix of proinflammatory cytokine. CCL16 exposure for only 24 h leads to formation of randomly distributed cell protrusions, and acquisition of the typical phenotypic features of mDC, a higher expression of costimulatory molecules CD80 and CD86, MHC class II molecules, the increasing expression of CD83 and CCR7. mDC/CCL16 are also strong stimulators in activating T cells in MLR. This provides functional confirmation of the maturation status of mDC/CCL16. Despite their mature characteristics, mDC/CCL16 are still able to take up FITC-dextran. These data reveal a new function for CCL16 in the immune response scenario. CCL16 significantly and quickly induces the maturation of DC, but they still maintain the ability to uptake Ag. These singular features could be important in the host defence against particular pathogens.

References

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Leptin promotes differentiation and survival of human dendritic cells and licenses them for Th1 priming

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Leptin is an adipocyte-derived hormone/cytokine that links nutrition, metabolism and immune homeostasis. Leptin is capable of modulating several immune responses. However, the effect of leptin on dendritic cells has not yet been recognized. As DCs are instrumental in the development of immune responses, in this study we evaluated the impact of leptin on DC activation. We demonstrated the presence of leptin receptor in human immature and mature DCs both at mRNA and protein level and its capacity to transduce leptin signalling leading to STAT-3 phosphorylation. We found no consistent modulation of DCs surface molecules known to be critical for their APC function in response to leptin. On the other hand, we found that leptin induces rearrangement of actin microfilaments, leading to uropod and ruffle formation. At functional level leptin up-regulates the IL-1 β , IL-6, IL-12, TNF- α

and MIP-1 α production. Coincident with this, leptin-treated DCs stimulate stronger allogeneic T cell responses. Furthermore, we found that leptin down-regulates IL-10 production by DCs and drives naive T cell polarization towards Th1 phenotype. Finally, we found that leptin protects DCs from spontaneous and UVB induced apoptosis. Consistent with the anti-apoptotic effect of leptin we observed the activation of NF- κ B and a parallel up-regulation of bcl-2 and bcl-xL gene expression. These results provide new insights on the immunoregulatory function of leptin demonstrating its ability to improve DC functions and to promote DC survival. This is of relevance considering a potential application of leptin in immunotherapeutic approaches and its possible use as adjuvant in vaccination protocols.

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Pro-angiogenic properties of alternatively activated dendritic cells

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Dendritic cells (DCs), in addition to inducing acquired immunity, are key modulators of inflammation. Here we show that human DCs matured in the presence of anti-inflammatory molecules like calcitriol, PGE2 or IL-10 (alternatively activated DCs, AA-DCs), selectively secrete vascular endothelial growth factor (VEGF), a potent angiogenic cytokine involved in tissue remodeling and repair during the late phase of inflammation. Conversely, VEGF is not produced by immature DC nor by DC activated in the presence of pro-inflammatory or immune signals, like LPS, TNF α , SAC or CD40 ligation. AA-DCs release 2 of the many VEGF spliced variants, VEGF165 and VEGF121 and, most importantly, AA-DC-derived VEGF is biologically active, as assessed by its ability to activate VEGFR-2 transfected cells. Finally, AA-DCs, but not immature or classically activated DCs, possess a VEGF-mediated pro-angiogenic activity *in vivo*, as shown by the chick chorioallantoic membrane assays performed in the presence of a blocking monoclonal antibody for VEGF, or an inhibitor of VEGFR-2 signalling. This study provides evidence that, with their pro-angiogenic activity, AA-DCs could contribute to the late phase of inflammation and tissue remodelling and could also play a critical role in pathological situations, such as chronic inflammation and cancer.

Role of mitochondria and reactive oxygen species in the differentiation process of human monocyte-derived dendritic cells

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Dendritic cells (DC) are potent antigen presenting cells with a unique ability in inducing T and B cell response as

well as immune tolerance. DC derive from bone marrow precursors and reside in an immature state in peripheral tissues where they exert a sentinel function for incoming antigens (Ag). Following an encounter with an Ag, DC undergo a maturation process that enhances their antigen presenting cell function and promotes their migration to the draining lymph nodes where they present processed Ag to naïve T cells. Recent studies suggest a role for Reactive Oxygen Species (ROS) as essential second messengers for DC response to several physiological stimuli. It has been established that the inhibition of the mitochondrial electron transport activity can induce ROS generation. Two sites of the mitochondrial respiratory chain, namely complex I and complex III, have been suggested to be the major source of ROS. Our preliminary results show 1) that the differentiation process of monocytes into dendritic cells is characterized by a 3-fold increase of endogenous respiration, and 2) that the presence of sub-saturating concentrations of rotenone, an inhibitor of the respiratory chain complex I, in the culture medium, inhibits DC differentiation process. Accordingly, rotenone-treated cells showed increased expression of CD14, a monocyte marker which is dramatically down-regulated during DC differentiation, and a decreased in CD1a expression, a marker of DC differentiation. In addition, rotenone-treated cells showed the expression of CCR7, a chemokine receptor involved in the trafficking and homing of DC to secondary lymphoid organs. Given the strategic localization of DC at the interface of innate and adaptive immunity, this study may provide the rationale for the identification of new targets in the regulation of DC functions.

Immune complexes affect differentiation, maturation and function of human monocyte-derived dendritic cells

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The interaction between receptors for the Fc portion of IgG (FcγRs) expressed by cells of the immune system and immune complexes (IC) triggers regulatory and effector functions. In this study, we evaluated the effect of IC on monocyte (Mo) differentiation into dendritic cells (DC). For this purpose, Mo were incubated with GM-CSF and IL-4 in the absence or presence of IC. On day six, the phenotypic profile of DC differentiated in the presence of IC (IC-DC) was significantly different from that of DC obtained in the absence of IC (DC): CD1a(%±SD): 85±6 vs 26.7±9; CD14(%±SD): 24±10 vs 45±8; MHCII(MFI±SD): 59±18 vs 120±37; CD68(MFI±SD): 241±70 vs 490±82, for DC and IC-DC, respectively (n=18, p<0.05). These results were confirmed by cytohistochemistry and suggest that IC inhibit the differentiation of Mo into DC favouring Mo differentiation into macrophage like cells. This effect of IC appeared to be irreversible, dose-dependent and more potent when IC were added at the beginning of the culture. On the other hand, IC neither altered the phenotype of already differentiated DC nor induced their maturation. Concerning functional activities, IC-DC displayed lower endocytic activity, did not mature in response to LPS and produced significantly lower levels of IL-12 as compared

with DC. Immune complexes are formed as the physiological consequence of the production of antibodies against foreign antigens or as the result of immune disorders. A decreased number of circulating DC has been reported in many autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus. We suggest that the inhibition of DC differentiation by IC may have a relevant role in the clinical manifestations of these diseases.

IMMUNE RESPONSE TO PATHOGENS

Evasion of host adaptive and innate immune responses by the HIV-1 Nef protein

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The HIV-1 virus has evolved several molecular mechanisms to evade the antiviral immune defenses of the host and establish a chronic infection. A major component of HIV-1 evasion of the CTL response is the viral Nef protein that, by downmodulating HLA class I molecules, protects infected cells from recognition and killing by virus-specific CTLs. Our studies with HIV-1-infected patients suggest a link between Nef-mediated HLA-I downregulation and the rate of disease progression. By structure/function analysis of patient-derived Nef protein variants, defects or duplication of the Nef proline-rich domain (PxxP) were found to be associated with defective or hyperactive phenotype on HLA-I downmodulation, respectively. The role of the PxxP domain of Nef on HLA-I cell surface reduction is independent from its two central prolines that mediate binding to SH3 domains and are required for Nef-mediated cell activation. However, Nef-mediated downregulation of HLA-I molecules on infected cells can alert NK cells which, unlike CTLs, preferentially lyse target cells with reduced cell surface HLA-I expression. Thus, to escape from the NK cell response, a clever strategy for the virus would be to interfere with the expression on infected cells of molecules that trigger NK cell effector functions. We addressed this issue and found that a cell can respond to HIV-1 infection by increasing the cell surface expression of HLA-I-related molecules (MICA, ULBP1 and ULBP2), that function as ligands of NKG2D, a potent activating receptor expressed on all NK cells. However, this response is counteracted by the Nef protein that is able to downmodulate NKG2D ligands, even in the absence of other viral proteins. This has important functional consequences, such as a reduction of NK cell-mediated lysis. Our findings strengthen the importance of Nef in the evasion of the host immune defenses against HIV-1 and highlight the need for drugs targeting Nef function.

Pathogenic B-cell responses to hepatitis C virus

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Chronic infection with hepatitis C virus (HCV) is associated with extrahepatic manifestations mostly caused by pathogenic B cell responses triggered by the virus. The