5. DISCUSSION

DCs are immunological “gatekeepers,” which are found throughout the body in a resting, immature form.

Upon exposure to danger signals, immature DCs become stimulated to mature and migrate to lymphoid tissues, where they are critical for the initiation of antigen-specific immune responses (Cella et al., 1997b).

Prior to such activation, immature DCs function to prevent inappropriate immune responses by promoting peripheral T cell tolerance (Steinman and Nussenzweig, 2002). Thus, the availability of immature DCs is key to maintaining immunological tolerance under normal conditions and to effective activation of antigen-specific immunity at times of challenge.

As the immature DCs population is being depleted continually by maturation and migration to maintain homeostasis, they must be replaced constantly from precursor cell populations.

The phenotypic and functional variations of DCs suggest they derive from multiple progenitor cell types. Myeloid lineage cells in the BM are known to give rise to precursor DCs, which enter the bloodstream and renew immature DC populations continuously within the tissues.

Peripheral blood monocytes are an abundant leukocyte subset, which can also serve as a DCs precursor population *in vivo* (Cavanagh and Von Andrian, 2002). However, monocytes are developmentally plastic, and most appear to differentiate into macrophages rather than DCs (Randolph et al., 1999).

The physiological signals that determine the course of monocytes differentiation are not fully characterized.

Experimental systems, reported in mouse models, based on the subcutaneous injection of fluorescent latex microspheres, interestingly demonstrated the differential potential of monocytes to differentiate into macrophages and DCs showing that monocytes that migrate to the draining lymph node has been shown to lead to DCs differentiation, whereas monocytes that remained at the injection site became macrophages (Randolph et al., 1998).

On the other hand, in humans it has not been defined which DCs subset originates from monocytes, and experiments regarding transendothelial migration of DCs generated from monocytes (Randolph et al., 1998)have not conclusively established which processes promote monocyte differentiation into DCs or into macrophages (Krutzik et al., 2005).

It is also known that exposure to particular cytokines can direct the course of monocytes differentiation: M-CSF stimulates macrophages differentiation, and GM-CSF, in combination with IL-4, leads to formation of immature DCs (Pickl et al., 1996; Piemonti et al., 1995; Romani et al., 1996; Sallusto and Lanzavecchia, 1994; Wiktor-Jedrzejczak et al., 1990).

However the physiological situations, in which monocytes are exposed to cytokines that direct DCs differentiation, remain unclear.

The present study demonstrates that human $CD4^{+}$ T lymphocytes can induce monocytes to differentiate into DCs upon antigen specific activation. DCs differentiation is induced in both antigen presenting monocytes, which establish an intimate interaction with specific T cells, and in bystander monocytes, which sense the cytokines released by activated lymphocytes. Monocytes differentiation into DCs occurs irrespective of the functional polarization of the T cells. Notably however, it was found that Th1, Th2 or Th0 cell clones drive monocytes to differentiate into DCs with diverse phenotype and functional capacity.

Autologous monocytes, co-cultured with T cells in the absence of antigen differentiate into $CD14^{low}$ $CD1^{-ve}$ macrophages. The phenotype of these cells do not differ significantly from the phenotype of macrophages derived from monocytes cultured with or without M-CSF in the absence of T cells.

This data suggest that the default differentiation pathway *in vitro* of monocytes leads to macrophages and, in addition, data indicate that non-activated T cells do not interfere with this process. On the other hand, PPD pulsed monocytes co-cultured with TCC specific for the same antigen differentiate into $CD14^{-ve}$ DCs.

The *in vitro* model described here is of particular relevance because it does not include the addition of any known monocyte differentiation factor into DCs, but reproduces the functional consequences on monocytes differentiation caused by a specific T cell activation, which follows antigen presentation.

This model is highly suggestive for reproducing the microenvironment of inflammatory sites in secondary immune responses.

Interestingly, the phenotype of these DCs varies according to the TCC polarization.

DCh2 showed a phenotype indistinguishable from reference DCk obtained culturing monocytes with recombinant GM-CSF and IL-4: they are immature $CD1^{+ve}$ cells that, upon LPS stimulation, turn into cells with a defined mature phenotype. DCh1 showed a

reduced expression of group I CD1 molecules and a more mature phenotype, which was not significantly modified by stimulation.

To investigate whether monocytes require cell-to-cell contact with TCC to differentiate into DCs, experiments using trans-well devices were performed.

Results indicate that differentiation occurs also in monocytes that are not in contact with activated TCC, and can be stimulated by the released cytokines diffusing through the trans-well. In addition, supernatants of anti-CD3 activated TCC without added cytokines act as DCs differentiation media for monocytes of all the tested donors.

These data do not exclude the possibility that a monocyte-T cell interaction might be involved, but clearly indicate that it is not required to induce monocytes differentiation into DCs.

Data also indicate that specific stimulation is required for T cells to be activated and release cytokines, which are responsible for monocytes differentiation into DCs. Moreover, the different phenotype of derived DCs reflects the cytokine secretion pattern of activated TCC. In fact, Th1 secreted cytokines and in particular GM-CSF, IL-3, TNF-α or IFN-γ have been previously described to induce monocytes differentiation into DCs (Ebner et al., 2002; Iwamoto et al., 2007) with characteristics different from those differentiated with cytokines secreted by Th2, such as GM-CSF, IL-4 or IL-5 (Sallusto and Lanzavecchia, 1994; Yi et al., 2007).

These data are in partial contrast with a recent published paper demonstrating the ability of NKT cells, but not of MHC class II restricted T cells, to induce monocytes differentiation into DCs (Hegde et al., 2007).

Since a pool of clones could not be representative of the general response *in vivo*, it was generated an additional set of Th1 and Th2 TCC specific for a different antigen (Parj1). Supernatants of activated Parj1 specific TCC are shown to induce monocytes differentiation as well as PPD specific TCC.

Moreover, it has been observed an *ex-vivo* DC differentiation using freshly isolated $CD4^+$ T cells and monocytes co-cultured in the presence, but not in the absence of a superantigen (SEA) as a stimulus.

Thus, a different *in vitro* setting could be responsible for the different results obtained in the cited paper (Hegde et al., 2007).

A previous paper suggested a possible role of activated T lymphocyte to induce monocyte differentiation into DC through the CD40 stimulation on monocytes by CD40 ligand (CD40L) (Brossart et al., 1998). However, the use of adherent instead of purified

monocytes and the lack of a formal demonstration of T cell associated CD40L involvement made the results of that paper non conclusive. However, even if this study demonstrate that soluble factors released by T cells are responsible for the differentiation of monocytes, it cannot be excluded that CD40-CD40L interaction may positively concur to the differentiation.

Next, the attention has been focused on the functional characteristic of DCs derived from monocytes sensing a Th1, Th2 or Th0 inflammatory microenvironment.

It is showed that DCh1 and DCh0 have a reduced capacity to prime naïve T cells in comparison to DCh2 and reference DCk. Moreover, in agreement with their reduced IL-12 and increased IL-10 synthesis, DCh1 and DCh0 showed a hampered capacity of inducing naïve T cell to acquire a functional polarization. In fact, the percentage of T cells capable of secreting IFN-γ and IL-2 is constantly reduced when these cells are used as APCs, in comparison to the percentage of polarized cells induced by reference DCk and DCh2. Interestingly, DCh1 and particularly DCh0 were characterized by the capacity to prime IL-10 secreting T cells with a possible regulatory role. The expansion of IL-10 secreting T cells seemed to be dependent on the presence of IL-10 itself (Levings et al., 2002). However, since both DCh1 and DCh0 secrete IL-10, but the expansion of IL-10 secreting T cells is mediated mainly by DCh0, other characteristics of DCh0, including their high TNF-α synthesis (Iwamoto et al., 2007; Menges et al., 2002) and CD86 expression (Weiner, 2001), must be involved and are now under investigation.

DCh1 and DCh0 are also showed to be less efficient than DCh2 and reference DCk to stimulate antigen specific TCC. TCC reproduce *in vitro* the function of experienced/memory T lymphocytes, which are characterized by requirements for their activation lower than naïve T cells (Viola and Lanzavecchia, 1996). Since the group I CD1 molecule expression in DCh1 and DCh0 is partially reduced in comparison to reference DCk and DCh2, it was not surprising to observe a decreased capacity of these APCs to present a lipid antigen to CD1-restricted TCC (De Libero and Mori, 2006a). However, the reduced ability of DCh1 and DCh0 to present antigen to MHC class II restricted TCC was unexpected, since their DR expression was higher than reference DCk and DCh2. This reduced activity may be in part attributed to a decreased uptake of antigens observed in these cells, in comparison to reference DCk and DCh2 and to their increased secretion of IL-10 (Groux et al., 1997; Liu et al., 2004), which probably accounts, together with other not yet known characteristic, to the observed disability of DCh1 and DCh0.

In conclusion, in this thesis is demonstrated that, upon antigen recognition, T lymphocytes secrete cytokines capable of inducing monocytes to differentiate into DCs. Although it is not easy and not always even possible to translate *in vivo* what observed *in vitro*, the phenomenon that is described herein is likely to occur in chronic inflammation sites where monocytes and lymphocytes are co-recruited (Tan and Coussens, 2007) and suggests that the differentiation of monocytes into DCs described in infected rodents (Leon et al., 2007) has the possibility to occur also in human, through the T cell mediated cytokine release. Together with T cell derived cytokines, it is possible to hypothesize that other micro-environmental factors related to the chronic inflammation and to its causes could contribute to the differentiation of monocytes into "inflammatory" DCs with diverse phenotypes and functions (Krutzik et al., 2005).

It is of relevance in this context that both NK and NK T cells have been shown to have the potential to induce monocytes to differentiate into DCs (Hegde et al., 2007; Zhang et al., 2007). However, the demonstration that DCh2, DCh1 and DCh0 have different capacity to induce the functional polarization of naïve T cells and to stimulate memory T lymphocytes is suggestive for the existence of a feedback control of Th1/Th2 immune responses orchestrated by monocytes-derived DCs.

Antigen/allergen specific immune responses characterized by a strong Th2 polarization, could be counterbalanced by the differentiation of DCh2 that, in turn, would prime new generation of antigen/allergen specific Th1 cells. On the other hand, the differentiation of DCh1/DCh0, which have a reduced capacity to prime new generation of Th1, and that are permissive to increase the priming of IL-10 secreting T cells with possible regulatory functions, associated with a disability to stimulate effector/memory T lymphocytes, could be instrumental to limit tissue damage in chronic inflammation sites (**Fig. 29**).

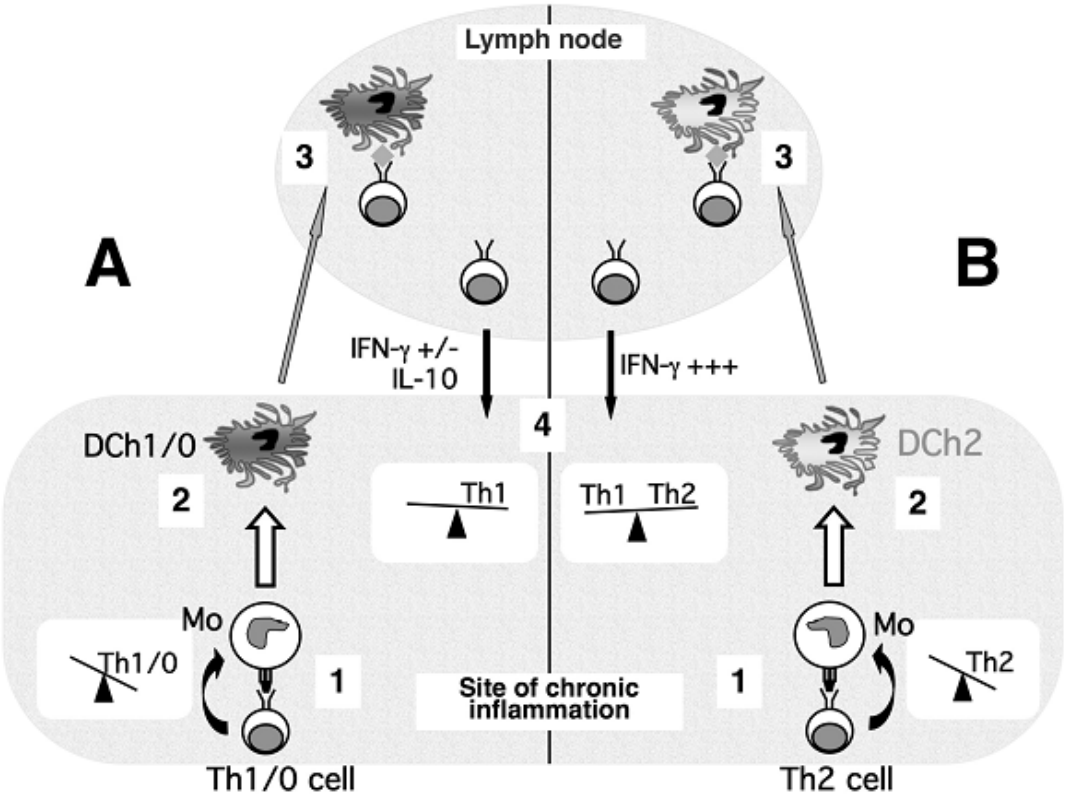

Fig 29. A schematic representation of the *in vitro* model described

The phenotype and function of DCs generated in such an inflammatory environment varies in relation to the Th1/Th0/Th2 phenotype of recruited lymphocytes, with a possible contribution to the regulation of the ongoing immune responses.
In the presence of a strong Th2 polarizing immune response, the differentiation of DCh2 could contribute to the priming of new generation of Th1 cells **(B)**.
On the other hand, the differentiation of DCh1 or DCh0, which have a reduced capacity to prime new generation of Th1 lymphocytes and to stimulate effector/memory T lymphocytes, and are permissive to increase the priming of IL-10 secreting T cells with possible regulatory functions, could be instrumental to limit tissue damage in chronic inflammation **(A)**.