

Dendritic Cells

Simon Skjøde Jensen and Jeppe Skytte Spicker at Bioneer A/S investigate the use of DCs in the development of immuno modulatory drugs

Dendritic cells are key regulators of the human immune system under both normal and pathological conditions. Dendritic cells are therefore prominent targets for therapeutic intervention in a range of immune disorders, as well as attractive targets for improving normal immune function in, for example, the development of new vaccine strategies, boosting the immune system during infection, or for immuno-compromised patients. This article presents and discusses the use of human dendritic cell models for predicting *in vivo* effects of novel anti-inflammatory drug candidates, probiotic microorganisms or natural extracts.

Dendritic cells (DCs) are part of the innate immune system upon which a pathogen challenge, for example, activates the innate and adaptive immune response. After a pathogen challenge, DCs migrate to the lymph nodes where they can activate lymphocytes to respond towards the infectious organism. In certain immune disorders, this process has been skewed towards an unfavourable type of response, resulting in allergic or autoimmune disorders.

DENDRITIC CELLS AS KEY PLAYERS IN IMMUNOLOGY

During the migration process of monocytes into the peripheral tissue, the monocytes differentiate into so-called immature DCs. In the tissue, the immature DCs exist in a steady state until challenged with a pathogen or activated by cytokines and chemokines secreted by other cells in the tissue environment. In tissues with inflammatory cytokines and chemokines, the DCs will migrate to the site of inflammation and mature into an active state. They are then termed inflammatory DCs (1). Thus, in inflammatory diseases like psoriasis, arthritis and IBD, inflammatory DCs are overabundant at the site of inflammation. Matured inflammatory DCs migrate to the

lymph nodes and activate the adaptive immune system, T-cells in particular.

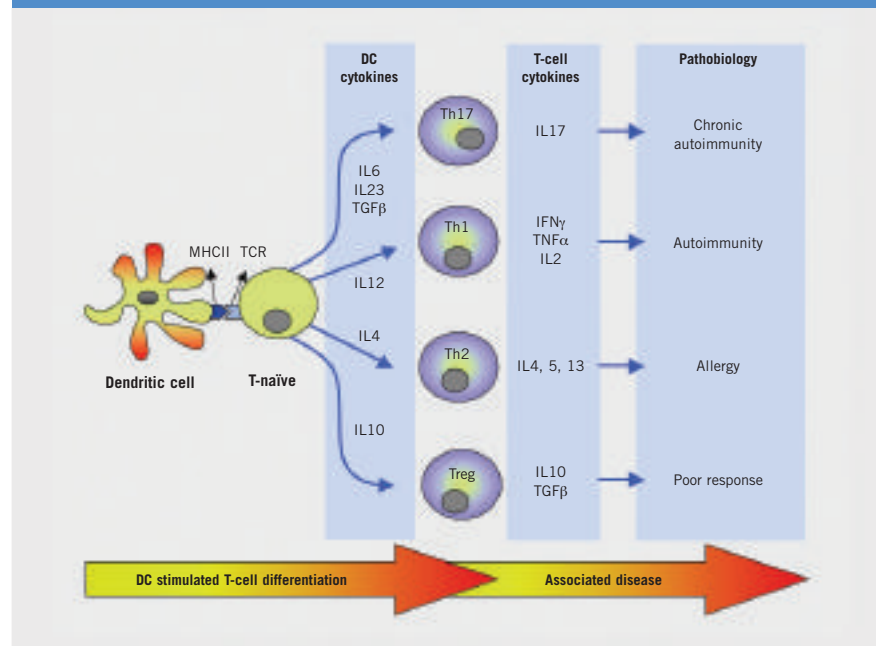
Four T-cell subsets are central for normal immune homeostasis, denoted either Th1, Th2, Th17 or Treg-cells (see Figure 1). Under normal immune homeostasis, these T-cell phenotypes are crucial for fighting

infections and for approving self-molecules that must be accepted by the immune system (2).

In inflammatory diseases such as psoriasis, arthritis and Crohns disease, there is a continuous development of

Figure 1: Key cytokines involved in the DC-mediated activation of naïve T-cells into different T-cell subsets

Each T-cell phenotype is involved in the normal immune homeostasis, but upon hyperactivation of T-cell lineages an immune disorder can develop (denoted in the box pathobiology).



In inflammatory diseases such as psoriasis, arthritis and Crohns disease, there is a continuous development of inflammatory DCs due to the high levels of cytokines and chemokines at the site of inflammation, with a subsequent activation of Th1-cells. Inflammatory DCs secrete hallmark inflammatory cytokines like TNF α , IL12 and IL23 which are important therapeutic targets in inflammatory diseases.

inflammatory DCs due to the high levels of cytokines and chemokines at the site of inflammation, with a subsequent activation of Th1-cells. Inflammatory DCs secrete hallmark inflammatory cytokines like TNF α , IL12 and IL23 which are important therapeutic targets in inflammatory diseases. In particular, neutralisation of TNF α function has been successful in the treatment of inflammation. Inflammatory DCs are also responsible for secretion of chemokines involved in recruitment of leukocytes to the site of inflammation (3). The chemokines and their receptors are currently highly interesting targets from a therapeutic point of view, since blocking

of ligand-receptor interactions could prevent further lymphocyte recruitment to the sites of inflammation (4). The DCs are central in the production of inflammatory cytokines and chemokines, which trigger the inflammatory process. Consequently, by targeting the upstream mediator of the immune response instead of the effector molecules, we may be able to treat immune disorders more efficiently (5). Suppression of DC function as an approach to escape the immune system has already been discovered by nature. The *Mycobacterium ulcerans* produces a toxin which blocks DC-mediated production of chemokines required for migration of

leukocytes to the site of inflammation. Furthermore, the toxin suppresses the ability of DCs to stimulate T-cell responses, thereby preventing activation of the adaptive immune system, and giving the bacterium a better chance to survive in its host (6).

EXPLOITATION OF HUMAN DENDRITIC CELL BIOLOGY FOR ANALYSIS OF IMMUNOREGULATORY COMPOUNDS

DCs are involved in autoimmune diseases, and are often the source of cytokines like IL12 and IL23, which are

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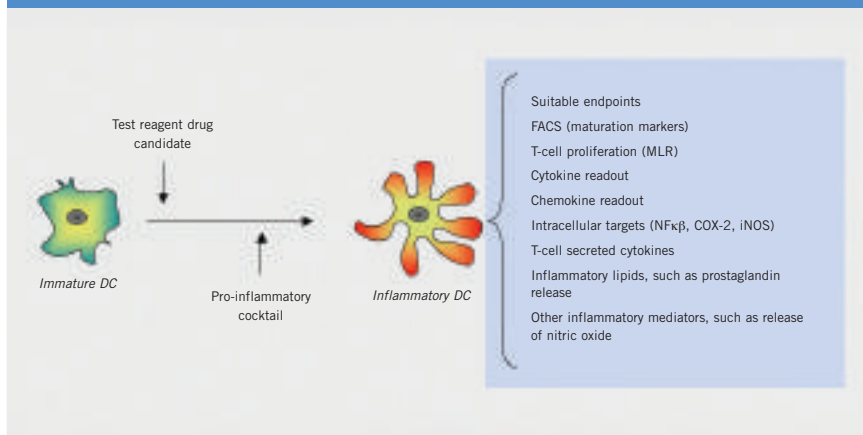
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Figure 2: The overall screening model based on human DCs

The model can assess general immune modulating effects using the test reagent alone and analysis of the end-points in comparison to controls. The anti-inflammatory screening model adds the pro-inflammatory cocktail after a few hours. Reagents which can suppress the inflammatory stimuli of the cocktail can be identified using the proper controls and end-points.



responsible for initiation and continuous enhancement of chronic autoimmune disease (7). The effector cells activated by DCs in autoimmune diseases like Crohns disease are mainly Th1 and Th17 cells (see Figure 1, page 46), which secrete cytokines like $TNF\alpha$, IL2, IL6, $IFN\gamma$ and IL17, some of which are the key targets for treatment of these diseases. Screening of immuno modulatory or anti-inflammatory compounds has traditionally been done using cell lines of monocytic or macrophage origin, or by expressing target proteins in established cell lines. However, these cell lines do not express the whole repertoire of innate immune receptors known as pattern recognition receptors, such as toll like receptors (TLR). Furthermore, cell lines can also be partially deficient in the natural cytokine and chemokine receptor signaling pathways. Accordingly, screening models based on cell lines might give false negative results, which may be detrimental for today's drug discovery process. DCs do express the natural pattern of receptors for innate responses and are reactive towards cytokines and chemokines involved in DC biology, which makes these cell types highly attractive for screening in mid to later stages of the discovery phase. Human DCs are emerging as a new essential tool for predicting safety or efficacy prior to entering clinical trials with DC-targeting drug candidates. Drawbacks for using DCs in screening are the costs associated with preparation of cells and intra assay variations, which mainly depends on heterogeneity of the monocytes

derived from the donors, and sub-optimal or inconsistent handling procedures. However, this variation can be minimised by using well defined and optimised differentiation procedures. Finally, the use of well defined pro-inflammatory stimulators, often termed cocktails, which initiate an inflammatory response *in vitro*, can be designed to stimulate the secretion of different cytokines, chemokines or lipid mediators being disease specific or relevant for evaluation of target-specific drugs directed at a specific target molecule or pathway in the DCs.

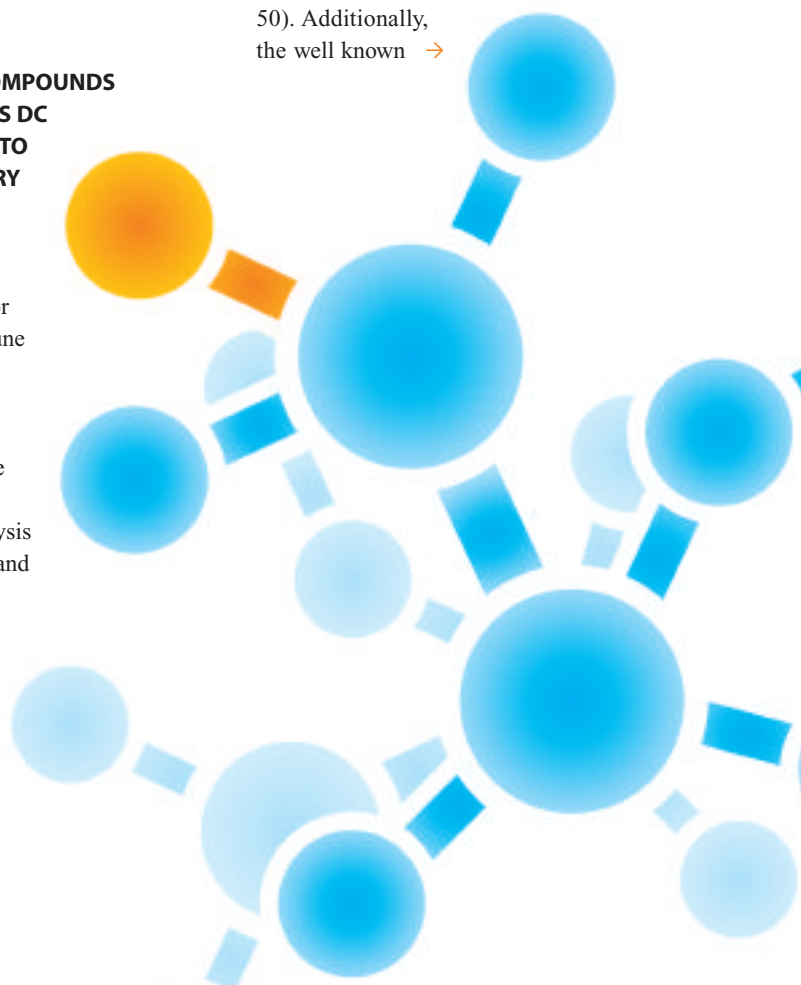
SCREENING OF COMPOUNDS ABLE TO SUPPRESS DC DEVELOPMENT INTO AN INFLAMMATORY DC PHENOTYPE

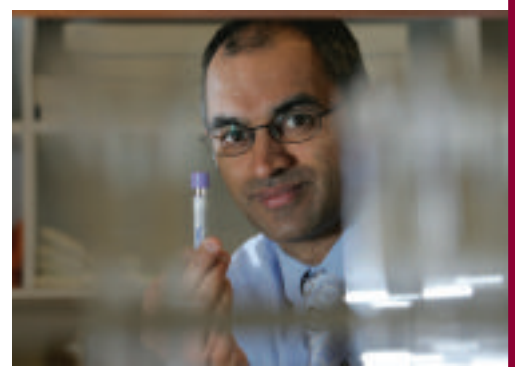
Human DC-based screening models for assessment of immune modulation of drug compounds, microorganisms or natural extracts have been developed and validated. This analysis gives an indication and a prediction of the reagent's ability to stimulate the immune system, including the direction in which the immune system may be modulated

(see Figure 2). We have combined this model with a novel screening method, in which we have defined pro-inflammatory cocktails consisting of combinations of innate immune stimulating components with pro-inflammatory cytokines and chemokines, components known to be involved in DC activation in different autoimmune disorders (see Figure 2) (8,9). The individual cocktails are optimised in order to stimulate DC-mediated secretion of key signaling factors such as IL12, IL23, $TNF\alpha$ and prostaglandin E2. By adding a test compound to the DCs before the pro-inflammatory cocktail, which mimics inflammation *in vivo*, we can analyse the anti-inflammatory potential of a test compound. If the test compound has the ability to prevent the cocktail-induced secretion of pro-inflammatory mediators or the development into a pro-inflammatory phenotype, the compound will most likely have a similar effect *in vivo*.

Human dendritic cell models can be used for a range of applications. This screening model has been successfully validated by demonstrating that drugs with a known function, such as dexamethasone, which is used in the clinic, are able to suppress the cocktail induced secretion of IL12 and $TNF\alpha$ (see Figures 3a and b, page 50). Additionally,

the well known →





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anti-inflammatory drug indomethasin can suppress prostaglandin E secretion from the DCs (see Figure 3c) providing further evidence that the DC model is functional. The model has also been useful for an assessment of potential immuno suppressive effects of microorganisms like probiotics, (bacteria with health-beneficial effects), mostly used in the functional foods industry. Probiotic bacteria of different type and origin for immune modulating effects in general have been screened, also for their ability to prevent development of inflammatory DCs using our defined cocktails. In Figure 3d, a probiotic strain is shown dose-dependently to be able to suppress IL12 secretion from these DCs treated with two different pro-inflammatory cocktails.


CONCLUSION

This article has described the importance of DCs in immune disorders. Furthermore, we have described how this sector is using DC-based screening models for identification of immuno modulatory drugs and ingredients. Treatment strategies targeting the

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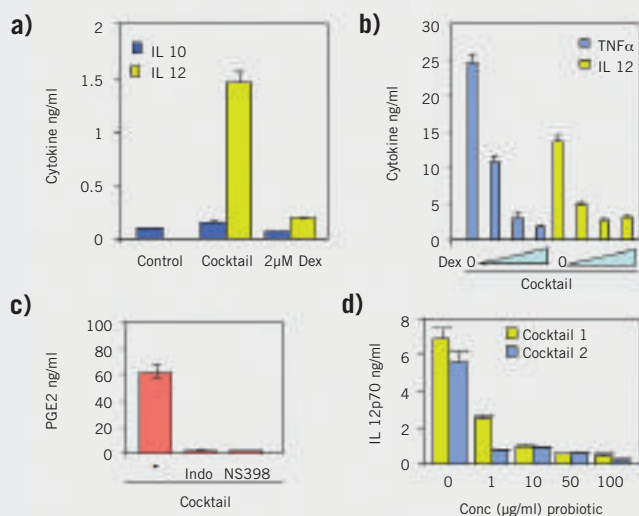


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tissue DCs with the ability to change an unfavourable condition from the path of inflammation towards a state of tolerance is a promising approach for intervention of inflammatory diseases. Such a treatment strategy might give rise to the next generation of immune modifying drugs, and certainly opens a new window of opportunities.

Figure 3: Results showing the ability of dexamethasone (Dex) to suppress cocktail induced IL12p70 and TNF α secretion from DCs (a and b)

The two COX-inhibitors indomethasin (indo) and NS398 can suppress cocktail induced prostaglandin E2 secretion (PGE2) (c). The probiotic strain B. bifidum BI98 can dose-dependently suppress cocktail induced IL12p70 secretion (d).



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