Pleiotropic functions of plasmacytoid dendritic cells in the pathogenesis of the rheumatoid arthritis

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ABSTRACT

Rheumatoid arthritis is a chronic autoimmune inflammatory disease with an unclear etiology. The disease is characterized by infiltration of synovial tissue with immune cells, among which there are dendritic cells that play multifaceted roles in the pathogenesis of the disease. Here we shall assume that plasmacytoid dendritic cells are able to change their phenotype under the influence of various stimuli, thereby modulating the course of the disease, contributing to both the development of exacerbations and the induction of remissions depending on the phenotype they have acquired. This property can be used to develop new methods of immunotherapy.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease with an unidentified etiology and a complex pathogenesis. RA is manifested by destructive polyarthritis, accompanied by infiltration of synovial tissue with various immune cells: T- and B-lymphocytes, macrophages, neutrophils, monocytes, dendritic cells [1]. The role of these cellular populations in the pathogenesis of RA is different. Of particular interest are dendritic cells, because of their ability to initiate an immune response.

In the joint synovial tissue of RA patients, two main subgroups of dendritic cells were identified: CD1c+ myeloid dendritic cells (mDC) and CD304+ plasmacytoid dendritic cells (pDC) [1]. mDC in RA are mainly pro-inflammatory: they increase and support the auto-inflammatory reaction [2], activating the T cells for the production of Th1, Th17 and Th2 cytokines [23]. Also, mDC produces high levels of chemokines (IL-16, TARC, MIG, IP-10) [23]. The functions of pDC are less clear. It has been shown that mDC can exhibit both pro-inflammatory and anti-inflammatory properties depending on various stimuli [23]. For the best understanding we performed the comparison between immunogenic and tolerogenic cytokines in relation to pDC (Table 1). Also, various studies establish both immunogenic (inflammatory) and tolerogenic (anti-inflammatory) pDC functions under normal conditions and in various diseases [5]. Therefore, a more extensive study of this subgroup of dendritic cells is needed to establish their functional significance in the autoimmune process, in particular in rheumatoid arthritis. Dendritic cellular vaccines based on mDC are widely studied the present time. The use of MPC-based vaccines is induced as immunogenic properties and tolerant is also a promising therapeutic method for the treatment of diseases to suppress and enhance immune responses (Fig. 1).

Hypothesis

Plasmacytoid dendritic cells of patients with RA under the influence of different stimuli can change their phenotype from the tolerogenic to the immunogenic, which can lead to the RA progression and contribute to the disease exacerbations. Change of the pDC phenotype to the tolerogenic may contribute to the development of remission.

1) According to present views, pDC have mostly an anti-inflammatory function in rheumatoid arthritis: the most of pDC derived from the synovial joint fluid and tissue samples of patients with RA demonstrate immature phenotype characterized by low expression of CD83 and DC-LAMP [3,4]. Immature pDC are able to induce anergy in T-lymphocytes, T-cell deletion and differentiation of regulatory T-cells (Treg) possessing tolerogenic properties [5]. Also, it was shown that mature pDC isolated from peripheral blood of patients with RA in remission demonstrate the high levels of 2,3-indoleamine dioxygenase enzyme (IDO) and contribute to differentiation of naive allogeneic CD4 + CD25- T-cells in the Treg producing IL-10 [6]. IDO is an enzyme that regulates the activity of T cells suppressing T-effector cells and promoting the differentiation of Treg [5]. Effects of tryptophan catabolism via IDO include the appearance of the regulatory phenotype in CD4 + CD25- naive T cells by TGF-β-induced Foxp3 expression [7]. It was also found that IDO acts as a signal protein in response to TGF-β inducing the conversion of naïve CD4 + T cells to Tregs [8]. Moreover, IDO acts as a regulator, which
Comparison between immunogenic and tolerogenic cytokines in relation to pDC.

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Immunogenic properties</th>
<th>Tolerogenic properties</th>
<th>References</th>
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<tbody>
<tr>
<td>IL-6</td>
<td>pDC are produced;</td>
<td>There are insufficient data available to verify the effect.</td>
<td>[9,11,20,24]</td>
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<td></td>
<td>enhances the differentiaton of the effector Th17 cells from naive T-lymphocytes and T-memory cells;</td>
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<td></td>
<td>probably, the effect on the TSLP level, which activates the mDC.</td>
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<td>IL-18</td>
<td>pDC are produced;</td>
<td>In accordance with the tolerogenic potential of pDC and mDC, the attraction of cells to the focus of inflammation helps reduce inflammation.</td>
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<td></td>
<td>enhances the expression of IL-1β and TNF-α;</td>
<td></td>
<td>[1,13-15]</td>
</tr>
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<td></td>
<td>attracts other pDC and mDC in the focus of inflammation.</td>
<td></td>
<td>Tolerogenic [5,23]</td>
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<td>IL-10</td>
<td>There are insufficient data available to verify the effect.</td>
<td></td>
<td>[6]</td>
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<tr>
<td>TGF-β</td>
<td>pDC are produced;</td>
<td>It promotes the differentiation of tolerogenic Treg.</td>
<td>Immunogenic</td>
</tr>
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<td></td>
<td>enhances the differentiation of Treg;</td>
<td></td>
<td>[11]</td>
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<tr>
<td></td>
<td>can promote Th17 differentation.</td>
<td></td>
<td>Tolerogenic [7,8]</td>
</tr>
<tr>
<td>TNF-α</td>
<td>pDC are produced;</td>
<td>There are insufficient data available to verify the effect.</td>
<td></td>
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<tr>
<td></td>
<td>enhances Th17 differentation;</td>
<td></td>
<td>[20,24]</td>
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<tr>
<td>IFN-γ</td>
<td>pDC are produced;</td>
<td>Can be associated with the induction of anti-inflammatory Treg.</td>
<td>Immunogenic</td>
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<td></td>
<td>attracts the TSLP level that activates the mDC.</td>
<td></td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td>together with IL-6, TGF-β enhances Th17 differentation.</td>
<td></td>
<td>Tolerogenic [16,17]</td>
</tr>
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</table>

stimulates the conversion of Treg in Th17-like phenotype: it was shown by in vitro experiment that antigen-activated effector T cells were activated by the conversion of Tregs in Th17-like phenotype, even with the involvement of interleukin-6 (IL-6) produced by activated pDC [9]. IDO suppressed the production of IL-6 in pDC and mediated its effects through the GCN2-kinase acting as a regulator [9].

In various animal models of RA deletion of the pDC subgroup by means of monoclonal antibodies was accompanied by an increase in the severity of arthritis and progression of morphological changes. In particular, an increase in CII-specific cell proliferation was shown in the models with pDC deletion [10].

2) The ability of pDC to exert pro-inflammatory properties has been demonstrated recently. It was shown that the pDC treated by CpG – oligodeoxynucleotide (CpG-ODN – are the short synthetic single-stranded DNA molecules activated the pDC-specific TLR9, with subsequent activation of various intracellular pathways) exhibit the ability to induce Th17 differentiation of naive T-cells through the production of IL-6, TGF-β, and IFN-α [11]. Th17 are important players in the pathogenesis of RA producing IL-17, IL-21, and IL-22. They recruit neutrophils and monocytes in the inflammatory focus, enhance production of proinflammatory cytokines such as TNFα and promote osteoclastogenesis that enhances bone damage [12]. Consequently, the ability of pDC to induce their appearance is a potentially proinflammatory factor contributing to the development of the disease. mDC in synovial tissue of patients with RA have an increased ability to activate the proliferation of T-lymphocytes, without a significant shift in the population of Th1 and Th17 type (predominant in RA) [26]. This fact demonstrates the role of other cells in a similar displacement, for example pDC. It was also shown that pDC from RA patients are able to produce IL-18 [1], which is associated with the local inflammation in synovial tissue in RA patients and related to IL-1β and TNF-α expression [13]. Moreover, it is possible for other DCs such as myeloid and lymphoid to be attracted in the focus of inflammation by IL-18 [14,15]. This fact, however, may be interpreted in different ways given the tolerogenic potential of pDC. It was shown that the activating potential of mDC in SF RA is associated with an elevated level of TSLP (thymus stromal limphopoetinum) [24]. The level of TSLP is determined by the synthesis of pro-inflammatory cytokines (such as IL-6 and TNF-α) [24], pDC can act as producers of such cytokines [17], as well as other factors that enhance their synthesis (eg, IL-18).

3) It was demonstrated that depending on the stimulus pDCs show different functional activity when the different classes of CpG-ODN
are activated (Table 2). Induction of CpG-A led to an increased synthesis of IFN-α and decreased degree of maturation, whereas CpG-B stimulation enhanced the maturation and production of IL-6 and TNF-α (by activating the intracellular pathway of NF-κB) [17]. This difference was associated with endosomal regulation because each ODN subgroup is contained within different endosomes, trig-
gging various intracellular activatory pathways [17]. Based on these data, it may be assumed that the CpG-A activation will lead to the formation of a tolerogenic phenotype of pDC (such pDCs were synthesized by IFN-I and had an immature phenotype which is as-
associated with the development of tolerance [22]). pDC-associated antigen presentation to T-lymphocytes led to the development of tolerance, since (in addition to the synthesis of IFN-I) they showed a low level of maturity associated with induction of tolerance in the antigen presentation to T-lymphocytes by immature pDC [22]. In contrast, CpG-B induction is likely to lead to a pro-inflammatory phenotype, due to the synthesis of pro-inflammatory cytokines IL-6 and TNF-α. In particular, it was shown that Th17 differentiation induced by CpG-ODNs was provided by an enhanced in vitro IL-6, TGF-β and IFN-α synthesis [11]. pDC maturation may be resulted in proinflammatory properties of pDC, activated by CpG-B.

One of the important properties of pDC in their functional rea-
arrangement concerns the main cytokine produced by pDC – IFN-I. IFN-
I is an important factor in the functional rearrangement of pDC. Based on the analysis of the present studies, it can be assumed that the ability of IFN-I to change the pDC phenotype depends on the spectrum of cy-
tokines that dendritic cells produce together with IFN-I. As mentioned above, the CpG-ODN treatment of pDC is able to induce Th17 differ-
entia-

tion [11]. It was shown that the secretion of cytokines IL-6, TGF-β together with IFN-α was required for such differentiation [11]. How-

ever, in another work, the CpG-ODN treatment of classes A, B and C of pDCs led to the differentiation of Treg cells producing IL-10, TGF-β, IFN-γ and IL-6. At the same time, activated dendritic cells did not de-
monstrate IL-10 and TGF-β expression [16]. This fact may indicate the ability of pDC to synthesize other antigenic factors that enhance Treg production. It is not known whether IFN was secreted by induction of Treg. However, it was found that CpG-A and CpG-C activation of pDC leads to IFN-I induction [17].

Treg induction was carried out by various classes of CpG-ODN (A, B and C) [16]. In contrast, Th17 induction was performed with the help of unknown class of CpG-ODN (probably CpG-C taking into account the spectra of the obtained cytokines [17]). In Moseman E.A. et al. [16] study the all classes of CpG-ODN were used for induction pDC Treg which subsequently led to the differentiation of regulatory T-lympho-
cytes. At the same time, the study of the influence of the different subgroups of CpG-ODN on functional activity pDC was shown that each class gives different results of pDC induction with its own spectrum of cytokines [17]. Different results of these studies were probably due to the fact that a lower dose of CpG-ODN (3 μg/ml) was used to induce Treg [16], whereas a higher dose (20 μg/ml) was used to generate different pDC responses [17]. Also, low concentrations of CpG-ODN (10 μg/ml) were used for Th17 induction [11]. Despite the fact that the levels of IFN-I were determined not in all the studies, there is reason to believe that interferon-I were synthesized in all cases pDC activation.

Several experiments on pDC activation with imiquimod (IMQ, li-
gand TLR7 in pDC) also demonstrate the different role of IFN-I in the formation of a particular cellular phenotype. In study of Nehmar R. et al. [18] it was demonstrated that imiquimod-activated pDC in mouse models of RA exhibited a tolerogenic effect that was associated with IFN-I. Also, it was suggested that IFN-III was synthesized in this ex-
periment (IFN-III has a pronounced anti-inflammatory effect, inhibiting neutrophil activity) [19]. However, another study showed that IMQ-
treatment of pDC led to the induction of Th17 from naive T-lympho-
cytes and memory T-cells [20]. This effect was accompanied by the secretion of proinflammatory cytokines IL-1β and -23p19, as well as IL-
6 and TNF-α by pDC after TLR7 activation [20]. At the same time, despite of IFN-I levels were not determined in this study it is possible to propose that IFN-I was also synthesized, because TLR-7 activation leads to the IFN-I expression [21]. A possible reason for the differences in the results is the different pDC sources. The tolerogenic properties were studied in pDC, which were obtained from tissues of mice with RA model [18]. Immunogenic properties were studied in pDC, which were obtained from healthy donors [20]. When IFN-I was secreted together with anti-inflammatory factors (it is even likely that IFN-I could act together with the tolerogenic factors that were expressed on the cell surface), it exhibited a tolerogenic effect. On the other hand, when IFN-
I was secreted together with pro-inflammatory cytokines, it led to an increase in the inflammatory response.

Based on the mentioned data, it can be assumed that IFN-I plays an amplifying role under the influence of other cytokines and that the functional orientation of pDC for tolerance and immunogenicity will depend not on IFN-I, but on the cytokines that are produced together with it or its concentration. Thus, it can be assumed that changes in the pDC phenotype in pa-
iients with RA can lead to the induction of pro or anti-inflammatory T
lymphocytes. This fact, on the one hand, can provoke an exacerbation and promote the progression of the disease and, on the other hand, induce a decrease in the autoimmune process and the development of remission. The influence of different stimuli can be crucial to establish the role of pDC in inflammation, which can change the course of the disease. It is also necessary to consider endogenous molecules as stim-
iuli. T-cell hyporesponsiveness upon (TSLP-primed) mDC stimulation in RA joints is partially dependent on PD-1/PD-L1 interactions, as PD-1 and PD-L1 are both highly expressed on SF T-cells and mDCs, respecti-
vely [25]. The possibility of induction of tolerogenic dendritic cells by
IDO expression upon contact with PD-1 was demonstrated [27]. IDO is
associated with a tolerogenic phenotype in pDC and overexpressed in
them in RA [6]. However, expression of PD-1 decreased with IL-7
production, which was associated with other inflammatory cytokines
[25]. The potential of pDC to induce the production of various pro-
inflammatory cytokines suggests that this cell population is capable to
influence on the escape from immunological tolerance. One of the ma-
ins issues is the possibility of a pDC transition from one phenotype to an-
tother. The study of this issue will contribute to an understanding of the
development and progression of rheumatoid arthritis. This results may

<table>
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<tr>
<th>Properties obtained by pDC from induction of CpG-ODN by</th>
<th>CpG-A</th>
<th>CpG-B</th>
<th>CpG-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokines</td>
<td>High IFN-I expression, expression of IL-6, TNF-α</td>
<td>Expression of IL-6, TNF-α; minimal expression of IFN-I</td>
<td>Expression of IFN-I; expression of IL-6, TNF-α</td>
</tr>
<tr>
<td>Molecules CD80/86</td>
<td>The lowest values of CD80/86 expression</td>
<td>High expression of costimulatory molecules CD80/86</td>
<td>Intermediate values of CD80/86 expression</td>
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<td>Functional mode</td>
<td>– activation of the immune response by high doses; – induction of tolerance by low doses.</td>
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References: [11,16,17].
be considered during development of the new therapeutic strategy for effective RA management.

Evaluation of the hypothesis

In order to test the hypothesis, it is necessary to carry out a series of experiments.

1) To check the reaction plasmacytoid dendritic cells from RA patients to stimuli that can lead to the formation of different phenotypes of pDCs. Plasmacytoid dendritic cells from RA patients should be isolated and affected by factors that alter the pDC phenotype, for example, CpG-ODN of three classes [17]. Based on the previous data, Cpg-GA should form a tolerogenic phenotype (Cpg-GA leads to formation of the less-differentiated pDC and IFN-I secretion). Cpg-B should exhibit immunogenic properties (Cpg-B mediates pDC maturation and proinflammatory cytokine IL-6 production).

The next step is the incubation of activated dendritic cells with naive T-lymphocytes. It is necessary to determine the phenotype of T-lymphocytes after incubation (it is probably that pDCs activated by Cpg-GA will promote the production of Treg and Cpg-B-activated pDCs will produce the induction of Th17 or Th1). In according to the previous data [16], the Cpg-ODN of all three classes will contribute to the development of Treg at low concentrations (Table 2). Therefore, it is necessary to establish the relationship between pDC phenotypes and Cpg-ODN concentrations. Also, it is necessary to determine the IFN-I level in all series of experiments. This will help establish the role of IFN-I in the induction of both the tolerogenic and immunogenic pDC phenotype (Table 1).

2) To determine the ability of activated pDCs to affect the course of RA. It is better to use the animal models of RA on this stage of the experimental study. Plasmacytoid dendritic cells from animal tissues with rheumatoid arthritis should be isolated and affected by factors that alter the phenotype of the pDC to a tolerogenic or immunogenic. Then introduce the activated dendritic cells back to the animal tissues. It is necessary to carry out the pDC activation in vitro to eliminate the influence of the environment on the organism Cpg-ODN activity and effect of Cpg-ODN on other cells involved in the disease process.

3) To record the progression of arthritis after the administration of activated immunogenic pDCs into the tissue. The experiment can be carried out on the animal model of rheumatoid arthritis. For the experiment, an arthritis model can be used after the anti-inflammatory therapy. Renewal of arthritis will simulate the transition of the disease from the remission stage to the exacerbation stage.

4) Verification of the ability of one pDC phenotype to acquire another phenotype. Cpg-activated pDCs of a single phenotype are treated with Cpg-ODN to produce another phenotype. For example, to treat Cpg-B-activated immunogenic pDCs by Cpg-A and detect changes in functional activity (the direction of naive T-lymphocytes differentiation, the spectrum of cytokines produced, Table 2). Changes in the tolerogenic pDCs after the treatment with Cpg-B are also recorded. The change in the phenotype of pDCs from the tolerogenic to the immunogenic and vice versa may be associated with alternating periods of remission and exacerbation.

Consequences of the hypothesis

If the hypothesis is confirmed, a potentially new role of pDC in the RA pathogenesis will be established: the regulation of the disease course. These functions of pDC can be considered in management of the other pathological states. Also, the dependence of the IFN-I effect on the spectrum of other produced cytokines will be established. This may contribute to the modification of IFN-I therapy for other diseases. For example, the use of IFN combinations with other cytokines to achieve an anti-inflammatory or pro-inflammatory effect (Table 1).

Dendritic cellular vaccines based on mDC are now widely studied. The properties of pDC in induction of immunological tolerance or immune response make this group promising in the development of cellular vaccines, on the one hand stimulating the immune response (therapy of oncological and infectious diseases), and on the other hand, weakening excessive immunoreactivity (allergic diseases, autoimmune pathology).

Conflict of interest statement

The authors declare no conflict of interest.

References

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