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REVIEW

Th1/Th2 CYTOKINE BALANCE IN ARTHRITIS

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Studies of the contribution of cytokines to the pathogenesis of rheumatoid arthritis (RA) have focused largely on monocyte-derived proinflammatory cytokines (1). Interleukin-1 (IL-1) and tumor necrosis factor α (TNFα), characterized as the most important ones, have been selected as significant therapeutic targets (2). The contribution of T cells to RA has been more a matter of debate and has been addressed in a number of reviews published in Arthritis & Rheumatism (3-5). A major issue has been—and remains—the remarkable contrast between the presence of a T cell infiltrate and the relative failure to detect definite markers of activity of these cells. Indeed, the low level of expression and production by the rheumatoid synovium of T cell-derived cytokines, as first described for IL-2 and interferon-γ (IFNγ), then extended to TNFβ and IL-4, were a primary reason to question the direct contribution of T cells to this disease (6).

The field has advanced recently by the description of new T cell-derived cytokines, which were assigned to various subsets of T cells. These subsets have, in turn, been associated with the development of different patterns of disease in mice and humans. Finally, modulation of the cytokine profile is now considered a therapeutic mean (7). In diseases such as RA, where a specific causative agent and/or antigen has not been identified, bypassing the antigen and acting on the cytokine balance might represent a way to control autoimmunity and chronic inflammation (8,9).

Th1/Th2 cytokine balance from mice to humans

In 1986, Mosmann and Coffman showed that mouse CD4+ T cell clones could be classified into distinct subsets according to their cytokine production pattern (10). Those studies established that IL-2 and IFNγ were produced by Th1 clones, whereas IL-4 and IL-5 were mostly produced by Th2 clones. Later, new cytokines were identified and were classified as Th1 for TNFβ and as Th2 for IL-6, IL-10, and IL-13. Simultaneously, a precursor (Th0) subset that produced IL-4, IL-2, and IFNγ was described (11). These precursor cells are themselves derived from virgin T cells (Thp), which secrete IL-2 following activation.

According to this simple dichotomy, Th1 cytokines favor T cell-mediated cellular immunity and cytotoxicity, delayed-type hypersensitivity (DTH), and they activate monocytes, which leads to the production of proinflammatory cytokines. Th2 cytokines favor B cell mediated-humoral immunity, induce IgE production with IL-4 and IL-13, activate eosinophils with IL-5, and deactivate monocytes, which leads to an antiinflammatory cytokine pattern (Figure 1).

Next, it was important to demonstrate that these patterns could also be recognized at a polyclonal level when looking at their tissue expression and production. Indeed, observations in animal contribution of Th1 cells to DTH and the contribution of Th2 cells to allergies and some parasitic infections. It was clear from the beginning that in the mouse models, the H2 genetic background had a major role in determining disease expression and its association with a particular cytokine pattern (11).

The same dichotomy was later applied to human T cells, starting with T cell clones and followed by application to various diseases (12,13). Thus, T cell clones from RA synovium were found to produce large amounts of IFNγ, but not IL-4, which led to their classification as Th1 cells (14,15). A similar conclusion was reached with CD4+ T cells from patients with autoimmune diabetes, thyroid diseases, and multiple...
Figure 1. The Th1/Th2 cytokine balance and biologic functions. A predominant Th1 profile is implicated in T cell-mediated immunity, whereas a predominant Th2 profile is implicated in B cell-mediated immunity. The opposite effects on monocyte activation are the basis for the control of chronic inflammation. IL = interleukin; IFNγ = interferon-γ.

Cellular Immunity
- Activation of T cells
- Activation of T cell cytotoxicity
- Activation of monocytes
- Increased production of proinflammatory cytokines

Humoral Immunity
- Activation of B cells
- Activation of eosinophils
- Deactivation of monocytes
- Decreased production of proinflammatory cytokines

Figure 2. The Th1/Th2 cytokine balance and diseases. A predominant Th1 profile has been associated with rheumatoid arthritis, Lyme arthritis, multiple sclerosis, diabetes, and thyroiditis. A Th2 profile, with the predominant contribution of interleukin-4 (IL-4) and IL-5, has been associated with allergic diseases and scleroderma. The predominant contribution of IL-10 has been suggested in pregnancy, systemic lupus erythematosus (SLE), and early human immunodeficiency virus (HIV) infection. IFNγ = interferon-γ.
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Figure 3. Control of the Th1/Th2 cytokine balance by monocyte-derived regulatory cytokines. Interleukin-12 (IL-12) is the critical monocyte-derived cytokine that induces interferon-γ (IFNγ) production and a Th1 profile through the activation of Stat-4. IL-4 is the critical cytokine that induces a Th2 profile through the activation of Stat-6. Solid lines represent stimulatory effects; broken lines represent inhibitory effects.

revealed when comparing the responses of monocytes from blood or synovium with these cytokines (21).

IL-4 and IL-13 share most of their activities, particularly their antiinflammatory properties (22). However, the effect of IL-4 on T cells appears more potent than that of IL-13. Furthermore, the 2 cytokines use the same multichain receptor, but in a competitive manner, as shown particularly with activated synoviocytes (23). The reason for the presence of the 2 molecules is unclear, and the use of one or the other cytokine for treatment would be difficult to justify.

New members of the Th1/Th2 cytokine balance. New cytokines have been isolated, and their classification is still pending. Among them, IL-17 is of particular interest in inflammation. This cytokine, which is produced by CD4+ T cells, acts on mesenchymal cells, such as synoviocytes, to induce the production of a proinflammatory pattern (24), with the induction of IL-6, GM-CSF, and prostaglandin E₂ production (25). Recent results indicate that this can be extended to other factors, such as leukemia inhibitory factor (LIF). These characteristics indicate that IL-17 can be classified as a type 1 cytokine. Moreover, its direct effect on synoviocytes represents a new way for T cells to induce inflammation, in particular, in association with low levels of monocyte-derived cytokines.

The Th1/Th2 cytokine balance in action

The dynamic pattern of the Th1/Th2 cytokine balance. The pattern of regulatory cytokine production is controlled by many parameters: the antigen structure (arthritogenic versus tolerogenic epitopes), the mode of administration (systemic versus oral), the antigen-presenting cells (dendritic cells versus B cells), and the cytokine and steroid environment (Th1 versus Th2) (26). This ability to switch between patterns may be useful when applying these concepts to treatment.

First, each Th cytokine subset induces its own production and favors the differentiation of naive T cells into the same subset. Thus IFNγ induces its own production and Th1 cell activation, whereas IL-4 activates Th2.

The second important feature of Th1 and Th2 cells is the ability of one subset to regulate the activities of the other. Both IL-4 and IL-10 are strong inhibitors of IFNγ production, and IFNγ inhibits IL-10 production (27) (Figure 1). Early results showed the direct contribution of the antigen. T cells from a given normal individual produce Th1 cytokines in response to mycobacterial antigens and Th2 cytokines in response to allergens (13). Furthermore, culture of normal T cells in the presence of either IFNγ or IL-4 results in the development of clones of either Th1 or Th2 type, respectively (28).

Recent results have shown that the balance can be manipulated with mitogens, without acting at the level of a specific antigen. This is a critical issue when considering the treatment of diseases of unknown specific origin, such as RA. These studies have indicated ways to induce a switch between the 2 opposite patterns. Human umbilical cord blood naive T cells activated by mitogens can be directed toward Th1 when cultured in the presence of IL-12 and anti-IL-4, and toward Th2 when cultured in the presence of IL-4 and anti-IL-12 (29). These findings demonstrate the critical role of IL-12 and that of IL-4 in the switch to Th1 and Th2, respectively (Figure 3).

The various cell-signaling pathways activated by these cytokines have been characterized. Such studies have focused on the role of the Stat transcription factors. IL-12 acts through the activation of Stat-4. Indeed, the inactivation of the Stat-4 gene in mice leads to a defect in IL-12 production, and thus of Th1 functions, combined with an increase in Th2 functions (30). Conversely, Stat-6 is critical for IL-4- and Th2-mediated events, as shown with Stat-6 knockout mice (31,32).

To monitor these changes, various surface markers, such as CD27 and CD30, have been proposed to
specifically identify these populations. However, additional studies have put to question their value for differentiating these subsets. Regarding arthritis, blood CD4, CD45RB<sup>dim</sup>, CD27- T cells have been characterized as the IL-4-producing subset (33). Their presence in the joint may represent an attempt to control inflammation (34). Recent studies have indicated a good correlation between the Th1 pattern and the expression of the B2 chain of the IL-12 receptor (29). Indeed IL-12 is critical for differentiation into a Th1 phenotype. Similarly, the transcription factor GATA-3 has been shown to be necessary and sufficient for Th2 pattern expression in mouse T cells (35). Additional studies are needed to determine whether these markers will be useful in the context of arthritis.

Molecules controlling the Th1/Th2 cytokine balance. Various molecules contribute to the control of Th1/Th2 development. The costimulatory molecules B7-1 (CD80) and B7-2 (CD86) expressed on antigen-presenting cells act as a second signal and control the differentiation of the 2 major subsets (36). Their ligands on T cells are CD28 and CTLA-4 (37). This second step of activation follows the first signal given by the interaction between the T cell receptor and the antigen major histocompatibility complex, which confers T cell specificity. CD28 costimulation was found to promote the production of Th2 cytokines by mouse T cells through an IL-4-dependent mechanism (38).

Initial results in the mouse have indicated that activation of the B7-1 pathway stimulates the Th1 subset, whereas B7-2 activation stimulates the Th2 subset. Conversely, blockade of B7-1 was able to control Th1-mediated diseases such as diabetes and encephalomyelitis (39,40). However, results in other systems indicate that this represents an oversimplification (41).

Application to humans has proven to be more difficult. However, the contribution of IL-4 appears to be critical. B7-2 stimulation specifically controls IL-4 production by naïve T cells, whereas both B7-1 and B7-2 can induce IL-4 production by memory T cells (42). In the absence of the regulatory properties of IL-4, T cells remain sensitive to other regulatory cytokines, such as IL-12, which induces a strong Th1 profile by inducing IFNγ production. In conclusion, the presence or absence of IL-4 appears to represent the critical control for the Th1/Th2 switch.

Th1/Th2 cytokine balance in arthritis

Expression in the synovium. This simple classification has been more difficult to reconstitute when looking at the cytokines produced by the synovium itself. Findings concerning the levels of these T cell–derived cytokines in RA have been contradictory and their specific detection difficult, despite infiltration of the synovium by many T cells (6,43). Although contradictory results have been published, the expression of IL-2 and IFNγ messenger RNA (mRNA) by RA synovium was not detected in one study even with the use of the most recent and sensitive methods (44). IL-4 protein and mRNA could not be detected in synovial fluid, synovial supernatants, or the synovium itself (45).

Cytokines such as IFNγ and IL-10 could not be easily detected in the supernatants of synovium pieces (19). However, staining for IL-10 was positive, and endogenous IL-10 was shown to be active, since an increased production of proinflammatory cytokines was observed in the presence of an inhibitory anti–IL-10 antibody (46). As indicated above, isolated synovial T cells do produce significant levels of IFNγ. Such production may be sufficient to induce potent local effects (Figure 4). The inhibitory effect of these 2 cytokines on each other may explain the absence of secretion when the secreting cells are in close contact (27), but the inhibitory component remains insufficient to control disease activity. Similar conclusions apply to TGFβ, which are also largely present in an active form in RA (45).

Th1/Th2 cytokine balance and the search for the cause of RA. A large body of work has tried to isolate the specific antigen(s) that contributes to RA. The lack of an identified causative agent in autoimmune diseases remains the major limitation to early diagnosis and treatment. In Lyme arthritis, the bacterial antigen is well known, and the histologic appearance of the synovium is very similar to that seen in RA. T cell clones specific for the bacterial agent were shown to produce large amounts of IFNγ with no IL-4, as in RA (47). Similarly, Th1 cells have been implicated in tuberculosis and leprosy (48,49). Along the same line, T cells infected by the retrovirus human T lymphotropic virus type I (HTLV-I) tend to produce an extended cytokine profile, combining T cell–derived cytokines of the type 1 pattern and monocyte-type cytokines. Such a profile could contribute to the rheumatoid-like clinical presentation observed both in humans and in transgenic mice for the Tax regulatory protein from HTLV-I (50).

Regarding the failure to detect a precise cause of RA, recent studies with a new transgenic model have indicated that destructive arthritis can be present in the absence of an intraarticular antigen (51). Whatever the
Th1/Th2 cytokine balance and the chronicity of RA. Increased migration of a large polyclonal population of T cell specificity results in the formation of the inflammatory lesion. Endothelial cell swelling, leading to the formation of high endothelial venules, is one of the earliest pathologic findings. These changes are associated with increased expression of adhesion molecules, leading to increased cell migration. Migration of Th1 cells, rather than Th2 cells, is facilitated through the use of the E and P selectins (52). Thus, expression of such adhesion molecules is mandatory for an increase in the migration of T cells in a non–antigen-specific manner. These findings in various animal models suggest that it is of therapeutic interest to act on the migration of inflammatory cells. Indeed, targeting of intercellular adhesion molecule 1 has been applied in the treatment of RA (53). To further refine such a concept, a more specific approach would be to block the migration of disease-inducing Th1 cells and favor that of the protective Th2 cells (54).

These changes in migration pattern are associated with differences between systemic and synovial sites. During chronic inflammation, migration of proinflammatory cells is increased, whereas that of protective cells is defective, leading to their relative accumulation in the bloodstream (52). In blood from RA patients, defects in IFNγ production have been reported (55). Results with very sensitive IL-4 assays indicated that activated RA whole blood cells produced more IL-4 than did controls, and with a higher IL-4:IFNγ ratio. Such findings are consistent with the increased production in blood as well as in activated peripheral blood mononuclear cell supernatants of soluble CD23, the production of which is enhanced by IL-4 (56). These results are in contrast to those found in the RA synovium, where a Th1 pattern is predominant.

Th1/Th2 cytokine balance as a therapeutic target in animal models

Direct targeting of cytokines. Results in various animal models have indicated that control of inflammatory diseases can be achieved in the absence of a direct action on the antigen. The regulatory role of IL-4 and IL-10 has been convincingly demonstrated in most, but not all, animal models of arthritis (57) (Figure 4). IL-10 production is found in the synovial tissue of DBA/1 mice shortly after the onset of autoimmune collagen arthritis, and animals receiving neutralizing anti–IL-10 antibodies display an accelerated disease onset and more severe
arthritis (58,59). Anti–IL-4 treatment around the time of the spontaneous onset of arthritis did not provoke enhanced severity (59), underlining a more dominant role of IL-10 in endogenous control, which is consistent with low levels of IL-4 in the arthritic tissue. However, combined treatment with antibodies against both cytokines resulted in the highest incidence and severity, indicating a significant contribution of IL-4 in disease control.

It is still unclear how much of the endogenous IL-10 is contributed to by Th2 lymphocytes. It seems likely that a large part of IL-10 in the arthritic synovial tissue is of macrophage origin. This may simultaneously explain the relative lack of IL-4 as compared with IL-10. Unlike IL-10, IL-4 is a selective product of T cells, with a minor contribution from local tissue mast cells. Consistent with observations in arthritis, IL-10 exerts a similar controlling role in other diseases, such as autoimmune encephalomyelitis, thyroiditis, and colitis (60). Besides IL-10, TGFβ also provides disease control, as shown by signs of spontaneous colitis both in IL-10– and TGFβ-deficient mice (61,62). Likewise, TGFβ may be derived from T cells as well as from tissue macrophages.

Although rather high levels of IL-10 and TGFβ can be found in arthritic tissue, further suppression of arthritis can still be achieved by exogenous application. Progression of established collagen arthritis could be ameliorated by daily treatment with IL-10 (63), but better suppression was found after combination treatment with IL-4 and IL-10. The mechanism of this synergistic protection is linked to a more optimal suppression of both TNFα and IL-1 production, and to a marked up-regulation of the IL-1 receptor antagonist (IL-1Ra)/IL-1 balance in synovium and cartilage (59). Moreover, there is evidence that IL-4 has a direct protective effect against chondrocyte-driven cartilage degradation (64).

**Induction of endogenous protective cytokines.** Although the therapeutic efficacy of daily IL-4/IL-10 treatment has now been proven in animal models of arthritis, it remains a challenge to avoid daily dosing and to find optimal ways to increase the endogenous production of suppressive cytokines at the site or to suppress the continued generation and propagation of Th1 cells. Strongly polarized T cell phenotypes are probably most relevant to chronic diseases that are characterized by continued antigen exposure, since it was found that reversibility of Th1 and Th2 phenotypes was lost after prolonged in vitro antigen stimulation (65). Despite major efforts in sophisticated animal models using T cell lines or clones, our understanding of regulation in conventionally immunized animals is still scant. Moreover, regulation seems rather different in early and late disease.

IL-12 is now recognized as a critical cytokine in the generation of Th1 cells. It is produced in large quantities upon activation of macrophages with bacterial stimuli. IL-12 can replace bacteria in adjuvant preparations, and this cytokine may be the natural link between intercurrent infections and relapses of autoimmune diseases. In murine collagen-induced arthritis, IL-12 markedly enhanced disease expression and severity when given during immunization (66) or at the time of expected onset, whereas anti–IL-12 treatment prevented arthritis onset (67). However, in established arthritis, regulation by IL-12 seems more complex. Exacerbations were noted after late anti–IL-12 treatment, apparently linked to IL-12–mediated induction of IL-10 (67). The latter phenomenon may represent a natural feedback mechanism that controls for largely skewed, and therefore pathogenic, Th1 responses. The bimodal activity of IL-12 will seriously complicate IL-12–directed therapy in arthritis.

**Induction of bystander suppression.** Of interest, activated Th2/Th3 cells may protect individuals from Th1-dependent arthritis through antigen-driven generation of IL-4, IL-10, or TGFβ. The principle of so-called bystander suppression through this set of cytokines provides a means of bypassing the lack of information on particular autoantigens involved in the disease (68) (Figure 5). Accepted ways to achieve Th2/Th3 responses are antigen dosing at mucosal surfaces, through the oral or nasal route, and stimulation with modified antigens (69,70). Oral dosing has a tendency to mainly generate Th3, TGFβ–dependent suppression. This was demonstrated by a loss of protection after treatment with neutralizing anti-TGFβ antibodies, whereas this pathway was still functional in IL-4–deficient mice (71). In contrast, studies with modified antigens provide evidence for the existence of an IL-4–dependent pathway, and it seems likely that multiple mechanisms may be operational (72). Of interest, treatment with nondepleting anti-CD4 antibodies during early antigen exposure caused a shift from Th1 to IL-4–producing Th2 cells (73), thus providing another means of manipulating T cell cytokine balance.

Although major research in this area has focused on understanding the immunoregulation mechanisms in autoimmune models such as encephalomyelitis and colitis (74), results in arthritis models have shown suppression of disease upon oral dosing with type II collagen (75,76). Although the principle of using an autoantigen
from the articular cartilage of the joint to create local generation of protective bystander cytokines at the site seems an attractive approach, the major hurdle is the influx of such bystander T cells in the synovial tissue. Oral or nasal dosing will generate Th2/Th3 T cells mainly at these local sites, and given the apparent lack of proper migration of Th2 cells as compared with Th1 cells into synovial tissues, which is linked to usage of different selectins (52), such Th2 cells may not end up in large quantities at the desired spot.

**Acting on the Th1/Th2 cytokine balance to control RA**

**Effect of exogenous cytokines.** Preclinical studies have been performed with synovium pieces and cell suspensions obtained after enzymatic digestion. Addition of exogenous IL-4, IL-13, and to a lesser extent, IL-10 to synovium pieces strongly reduced the production of proinflammatory cytokines, whereas the production of IL-1Ra was enhanced (45,46,77). Furthermore, addition of bone pieces reduced bone resorption through an apparent effect on osteoclast activity and survival (78). A similar conclusion was reached concerning cartilage degradation (79) (Figure 4). It is of interest to note that although IL-4 reduced the production of IL-6 by synovium pieces, it enhanced its production by synoviocytes (80). Under the same conditions, the production of LIF was inhibited (81). These findings underline the complexity of the picture when considering results obtained with isolated cells outside their in vivo environment.

These in vitro results provide the rationale for treatment (82). The most simple approach is the administration of exogenous cytokines. Nevertheless, their half-life is very short, and such molecules will bind to specific receptors expressed on cells all over the body, preventing the diffusion of the molecules to the inflammatory site. Local administration would not be difficult,
but it will be limited by the number of affected joints. Preliminary phase I trials have started in RA, using the systemic administration of IL-10 and IL-4.

Administration of cytokine by gene therapy has proven to be successful in animal models of arthritis (83), but the precise local control of such genes remains to be resolved. Another means could be the ex vivo stimulation of circulating lymphocytes with exogenous cytokines with reinjection of the cells. Such a procedure has already been used with IL-2 to obtain lymphokine-activated killer cells in the treatment of cancer. Ex vivo activation with Th2 cytokines can be a way to induce the differentiation of uncommitted or Th1 cells into Th2 cells.

Induction of the endogenous production of protective cytokines. Induction of a remission or cure of RA has been observed in a number of clinical conditions in the absence of an obvious action at the level of the cause (9). These conditions are important to consider, since it is tempting to attempt to reproduce such modulation for a longer period. The most obvious situation is the remission of RA that is observed during pregnancy and the flare that follows delivery. In this situation, T cell-mediated immunity—rejection of the fetus—must be down-regulated, whereas the fetus has to be protected with maternal IgG (84). These 2 components suggest the contribution of factors such as TGFβ and IL-10. Indeed, high levels of expression of IL-10 at the placental interface have been observed in the mouse (85). More importantly, the improvement in RA appears to be directly related to the degree of maternal–fetal disparity, which leads to an increased need for fetal tolerance (86). Similar observations have been made for the side effects of slow-acting drugs (87), for early human immunodeficiency virus infection (88), and for allogeneic bone marrow transplantation (89) (Figure 2). These conditions appear to be associated with a Th2 cytokine profile (8,90) (Figure 2).

Whatever the mechanism, such observations indicate that even longstanding inflammatory disease can improve without antigen-specific intervention. The next logical step is to reproduce these situations in vitro and in vivo. Although the clinical data from the first small trials of type II collagen dosing in RA patients looked promising (91), more extended studies did not confirm efficacy (92), and we are yet at the beginning of exploring focused manipulation of local Th1/Th2/Th3 balances. Nevertheless, induction of bystander suppression by oral tolerance could represent a simple way to control inflammation.

It should be kept in mind that excessive Th2 switching may be harmful. Excesses of IL-4 and IL-13 could lead to allergy and asthma. An excess of IL-10 could favor B cell/plasma cell activation with increased autoantibody production. Such events may contribute to some extraarticular manifestations such as rheumatoid vasculitis or lymphomas that are seen during the course of RA.

Conclusion

The concept of cytokine balance in arthritis is appealing since it leads to treatment even if the cause of the disease cannot be identified. This implies early treatment before destruction in patients at high risk. The genetic and hormonal backgrounds are obviously linked to RA severity. Recent findings suggest that the invasive pattern of RA synoviocytes is associated with, and may be the consequence of, irreversible somatic mutations (93). If this is the case, then endogenous control of such proliferation may remain limited when the disease becomes chronic. Taken together, these factors strongly point to the need for better early diagnosis and prognostic markers to ensure early treatment. In this situation, acting on the endogenous regulatory cytokine balance may represent a more natural way to prevent the consequences of chronic inflammation.

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