Main Role of Cytokines in Autoimmunity*

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The main function of the immune system is defending against foreign antigens. For this purpose the body responds by two mechanisms – the innate or natural response, whereby phagocytic cells and natural killer cells release inflammatory mediators and cytokines, and the acquired or adaptive response, which involves the proliferation of antigen-specific T and B cells. Autoimmunity is defined as the inability of the immune system to distinguish between self and non-self. The immune system has the capacity to maintain a state of equilibrium, although it responds to a diverse array of foreign antigens and despite its permanent exposure to self-antigens [1]

During the last few years, much progress has been made in the understanding of processes that lead from a normal autoimmune state, in which no clinical manifestations exist, to an autoimmune disease. Autoimmunity can be destructive or protective depending on the tissue context. It has been hypothesized that following an infection, immune response spreads to tissue-specific autoantigens in genetically predisposed subjects, eventually determining the progression to disease. Molecular mimicry between microbial/viral and self-antigens could, in some instances, initiate autoimmunity. Local release of inflammatory cytokines following infection probably plays a pivotal role in determining loss of tolerance to self-autoantigens and the pathogenic activation of autoreactive cells [2].

It is becoming increasingly apparent that T cell tolerance to self-antigens cannot be accounted for solely by intrathymic clonal deletion or induction of anergy in peripheral T cells. Several experimental models have been described in which expression of the pathogenic capacity of potentially self-reactive T cells was actively prevented by other T cells with an immunoregulatory role [3].

Primary exposure of naive CD4+ T cell to antigen results in differentiation to a defined helper subset. The preferential development of a particular Th- cell subset has been correlated directly with either susceptibility or resistance to certain disease states. Various factors have been shown to influence the differentiation of particular Th response, including antigen type, antigen dose, the type of antigen-presenting cell, and the presence of cytokines. The primary helper types are Th1 and Th2 and are characterized by distinct patterns of cytokine secretion. In the present review I will discuss the current knowledge concerning cytokine participation in the pathogenesis of autoimmune diseases by analyzing evidence for their participation in these diseases. Each disease exhibits some similar and some distinct features, some of which are shared with other autoimmune diseases [4].

Although both CD4+ and CD8+ T cells secrete cytokines, CD4+ cells secrete them in significantly larger quantities. CD4+ T cells can be further subdivided by the patterns of cytokine released: Th1 cells produce interleukin-2 and interferon-gamma, which are critical for cell-mediated immunity, whereas Th2 cells produce IL-4, IL-5 and IL-10, which promote antibody production and humoral immunity [4]. When naive T cells first exit the thymus, they can secrete both Th1 and Th2 cytokines and are termed Th0, but as these cells encounter antigen and become memory cells their cytokine patterns become fixed as either Th1 or Th2. Responses by a given T cell clone to a particular antigen tend to fall into one or the other pattern, especially in rodents where T cell responses are more rigidly fixed than in humans. The exclusive expression of Th1 and Th2 responses arises in part because these T cell subtypes suppress each other's function. This cross-regulation has given rise to the "Th1/Th2" paradigm, in which a dominance of pro-inflammatory Th1 cytokines causes destruction of target tissues and a loss of tolerance, whereas the dominance of Th2 cytokines suppresses the Th1 response and promotes tolerance [5].

Among the factors known to play an important role in determining the Th1-Th2 balance are antigen and co-stimulation. Both low and high antigen concentrations tend to induce Th2 responses preferentially, whereas intermediate doses induce Th1 responses. The mechanism(s) responsible for these effects is poorly understood. It is possible that certain concentrations of antigen induce a state of tolerance that preferentially shuts off Th1 responses.

During the presentation of antigens, the type of APC also has a profound effect on the resultant immune response. Langerhans cells and dendritic cells induce Th1 proliferation,

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IL = interleukin
APC = antigen-presenting cell

whereas B cells tend to induce a Th2 response. A major factor in this dichotomy in APC function is the elaboration of particular cytokines upon antigen encounter [4,5].

Th1 cells secrete IL-2, tumor necrosis factor-alpha and INFγ and are involved in cell-mediated inflammatory responses. Th2 cells secrete IL-4, IL-5, IL-6, IL-8, IL-10 and IL-13, and favor humoral responses and/or allergy [6]. An important feature of these TH cell subsets is the ability of one subset to regulate the activities of the other. The balance between these cells can play a major role in the type of disease manifestation observed. This balance depends on many factors, including the antigen structure, the antigen-presenting cells and the cytokine environment. This feature is critical for the understanding of adverse effects induced by cytokines. There is much evidence that the cytokine products of each individual Th- cell subset inhibit both the differentiation and effector functions of the other. For example, IFNγ has been shown to prevent Th2 cell proliferation, whereas IL-10 inhibits the synthesis of Th1 cytokines [6,7]. Therefore, the emergence of a Th2-type response typically results in the inhibition of Th1 differentiation and the down-regulation of Th1-mediated immune responses.

Almost all cytokines are pleiotropic molecules showing multiple biological activities. Furthermore, several cytokines often have overlapping activities, and a single cell frequently interacts with multiple cytokines with similar responses. As a consequence, one factor may functionally replace another altogether or at least partially compensate for the lack of another mediator. Since most cytokines have ubiquitous biological activities, their physiological significance as normal regulators or in pathological situations is often difficult to assess.

Th1/Th2 cytokines

Since Mosmann and colleagues reported the existence of two different CD4+T cells based on the cytokines they produce, the Th1/Th2 model has evolved to encompass several newly discovered cytokines and major new functions.

The term Th1 cytokines and Th2 cytokines refers to the patterns of cytokines secreted by two different subpopulations of murine CD4+ T cells that determine the outcome of an antigenic response toward humoral or cell-mediated immunity. Numerous cells other than CD4+ T cells have been shown to be capable of producing Th1 cytokines and Th2 cytokines. These cells include CD8+ T cells, monocytes, natural killer cells, B cells, eosinophils, mast cells, basophils, dendritic cells and others. Th 1 cytokines include IL-2, IFNγ, IL-12 and TNFα, while Th2 cytokines include IL-4, IL-5, IL-6, IL-10 and IL-13 [16,17]. The most potent cytokine inducer of Th2 cells is IL-4. Several types of immune cells can produce IL-4 in the initial immune response, including NK1.1+ CD4+ T cells, T cells, CD8+ T cells, and/or mast cells [8,9].

As previously mentioned, IL-10 is known to inhibit the induction of Th1 immunity and allows for the elaboration of a Th2 response. Since IL-10 can be produced by certain APCs [18], it has been hypothesized that under the influence of transforming growth factor-beta, the preferential production of IL-10 by the APC leads to the development of Th2 immunity in autoimmune diseases. In addition, it has been hypothesized that the autoregulatory effects if IL-10 on APC function might be a critical factor in the ability of an APC to present antigen in a suppressive manner [10].

In direct contrast to IL-4 or IL-10, IL-12 strongly induces the development of Th1 cells. Activation of monocytes/macrophages usually results in the elaboration of pro-inflammatory IL-12 cytokine [11]. The effect of IL-12 is twofold. One effect is the direct stimulation of Th1 differentiation. Another effect is the stimulation of IFNγ production by T and natural killer cells. INFγ is a potent inducer of Th1 immunity both by the additional stimulation of IL-12 secretion and the inhibition of IL-4. Th1 cell development depends on IL-12 produced by APCs. The interaction of the CD40 ligand on T cells with CD40 on dendritic cells results in very high production of IL-12 [12], thus favoring the development of a Th1 response. Because of the potent pro-inflammatory and immunoregulatory functions of IL-12, the immune system has developed feedback mechanisms for antagonizing its action. IL-10 negatively regulates IL-12 p40 transcription. In B cell lines, NF-κB appears to play a crucial role in the regulation of IL-12 p40 production. Molecular analysis of the promoter region of the human p40 gene has also identified a member of the Ets family of transcription factors as a major regulatory factor [13].

Another exogenous factor also influencing the development of undifferentiated CD4+ T cells towards either the Th1 or Th2 phenotype is TGFβ [14] Murine Th2 cells, but not Th1 cells, also express P600, the human counterpart of which has been identified as IL-13. A novel cytokine inducing the synthesis of IFNγ in Th1 cells has recently been identified as IL-18 [15]. Th1 and Th2 cells not only produce a different set of cytokines, but also appear to express different activation markers preferentially. CD30, a member of the TNF receptor superfamily, is mainly expressed by Th2-like and T cytotoxic cells, whereas lymphocyte activation gene 3 (LAG-3), a member of the immunoglobulin superfamily, preferentially associates with Th1-type cells. Recently identified was a stable cell surface marker (STL2) expressed on Th2 but not Th1 [16].

Th1-type and Th2-type responses in humans could play an important role in certain diseases. Th1-type responses are involved in the pathogenesis of organ-specific autoimmune disorders and acute allograft rejection, as well as in some chronic inflammatory disorders of the gastrointestinal tract,

IFN = interferon
TNFγ = tumor necrosis factor-alpha

TGF = transforming growth factor

such as gastric antritis and Crohn's disease. By contrast, allergic reactions involving immunoglobulin E and mast cells result from the development and activation of allergen-specific Th2 cells. It appears that different types of helper cell populations resembling those observed in mice are found also in humans. However, the differences in cytokine expression seem to be quantitative rather than qualitative.

Cytokines in the pathogenesis of systemic lupus erythematosus

Systemic lupus erythematosus is characterized by multisystem involvement and autoantibodies to nuclesome, cytoplasmic and cell surface autoantigens. The mechanism responsible for the breakdown of self-tolerance is unknown. This disease has a multifactorial pathogenesis with genetic and environmental precipitating factors. The immune dysregulation that characterizes this disease is complex, but it has two main characteristics. One is B lymphocyte hyperactivity and immunoglobulin repertoire changes, causing both increased spontaneous production of immunoglobulins and autoantibody production [17]. The mechanisms whereby B lymphocytes remain inappropriately activated for years during SLE are still poorly understood and may involve several agents acting in concert: genetic background, environmental factors such as drugs, hormonal influences, viruses and abnormal production of cytokines [18,19]. The other is an impaired cell-mediated immunity. This includes decreased T cell proliferative responses in autologous mixed lymphocyte reactions or following stimulation with mitogens, defective co-stimulatory signals and several defects in the production of cytokines. The impaired cell-mediated immunity results from both T lymphocyte and APC cell dysfunctions [19].

Cytokine regulation of SLE

Since 1982, when we reported a defect in the production of and response to IL-2 by mononuclear cells from SLE patients, the research on cytokines in this disease has become the subject of numerous studies [20–22]. Cytokines have been suggested to play an important role in the immune dysregulation observed in SLE patients and murine lupus-prone strains. Some of the T cell abnormalities found in SLE patients can be attributed to decreased activity of IL-2. Whether due to an intrinsic T cell alteration or not, a defect in the IL-2 regulation is present in patients with SLE and – causal or not – contributes to the complex disturbances of the immune system of SLE patients.

Although the origin of this abnormality is unknown, several mechanisms have been proposed. The first is the primary defect of CD4 cells. The second is the suppressive effect of the activity of IL-2 by CD8 cells, by specific antibodies against IL-2 or by other cytokines. The third is exhaustion of T cells due to their activation *in vivo*; and the fourth is the deficiency of other cytokines that participate in T cell activation. It was later shown that this was not an intrinsic defect, since it recovered after the cells were rested [23]. At that time it was also shown that SLE monocytes also have defective production of and response to IL-1 [21–23].

Multiple cytokine-mediated alterations have been demonstrated in SLE patients. Thus, abnormal (increased or decreased) production of IL-1, TNFα, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, TGFβ and IFNγ have been reported [24]. Moreover, some of these cytokines (IL-1, TNFα, IL-6 and IFNα) have been found in renal tissue of these patients, suggesting a local pathogenic effect [25].

B lymphocytes from SLE patients spontaneously produce IL-6 and constitutively express IL-6 receptors [41]. *In vitro* inhibition of this autocrine loop by anti-IL-6 receptor antibodies decreases the spontaneous production of autoantibodies. Anti-TNFα antibodies also decrease production of immunoglobulins by cultured peripheral blood mononuclear cells of SLE patients, but circulating TNFα is not detected in such patients. A correlation has been shown between the serum levels of TNFγ receptors, IL-1 receptor antagonist and clinical disease activity. Also, an overexpression of TNFα mRNA has been found in bone marrow cells from these patients [26]. The elevated levels of TNFα and IL-10 observed in SLE patients reflect the hyperactivation of cells that are responsible for the overproduction of autoantibodies. Once induced, these cytokines act as pro-inflammatory effectors through their biological properties, including the stimulation of a cascade of other soluble factors that amplify the initial stimulus and lead to the progression of the disease.

Abnormal IFN production has been found in SLE patients. This cytokine has pleiotropic effects on the immune system, and its defective production can induce autoimmune manifestations. It has a role in the up-regulation of major histocompatibility complex class II molecules and in this way contributes to the pathogenesis of autoimmunity. The B cell hyperactivity found in SLE and in other autoimmune diseases may be related to a generic increase in Th2 cytokines, an increase shown to be due to IL-4, IL-6 and IL-10. However, it was recently shown that IL-6 production in SLE could be due to hyperactivity of cells other than T cells, and that increased IL-10 production is actually due to monocytes and B cells rather than to T lymphocytes [27]. These autocrine and paracrine effects of IL-10 might be crucial to the B cell hyperactivity, and particularly to the autoantibody production. IL-10 is a potent stimulator of B lymphocytes and stimulates the production of anti-DNA autoantibodies by PBMC cells from SLE patients [28]. It is also a potent inhibitor of both antigen-presenting cell and T lymphocyte functions. Increased production of IL-10 could thus explain the two main characteristics of the immune dysregulation of SLE.

Immune dysregulation partially mimicking that of SLE has

SLE = systemic lupus erythematosus

PBMC = peripheral blood mononuclear cells

been described in healthy relatives of SLE patients. Some of these family members display autoreactive B lymphocyte hyperactivity, although the autoantibodies produced are of low affinity and are not pathogenic. A larger proportion of the relatives display impaired cell-mediated immunity, decreased IL-2 production and polyclonal B lymphocyte hyperactivity. Relatives of SLE patients display a dysregulation of IL-10 production similar to that of SLE patients. This finding strongly suggests that IL-10 gene dysregulation might belong to the background predisposing to the disease rather than representing a simple marker of immune activation [29].

A recent evaluation was conducted regarding the safety and efficacy of administering an anti-IL-10 monoclonal antibody to SLE patients with active and steroid-dependent disease. Treatment was found to be safe and well tolerated. In all patients, cutaneous lesions healed and joint symptoms were ameliorated. This is the first report of IL-10 antagonist administration in humans. It demonstrates the involvement of IL-10 in the pathogenesis of SLE and indicates that the use of IL-10 antagonists may be beneficial in the management of refractory SLE [30].

IL-12 is another cytokine that appears to play a role in polyclonal B cell activation observed in SLE. Its presence in mononuclear cell cultures is able to down-regulate the spontaneous immunoglobulin production; this inhibiting activity does not seem to be mediated through IFNγ secretion. Recently reported was the decreased production of lymphocyte-derived TGFβ in SLE, which cannot be normalized by the addition of IL-2 and TNFα or by antagonism of IL-10. Therefore, abnormal production of each of these cytokines in SLE could be important in the perpetuation of B cell hyperactivity [31].

Conclusions

The many specific activities of individual cytokines have been the basis for current concepts of therapeutic intervention, particularly for the treatment of organ-specific autoimmune diseases and tumor therapy. Applications involve the support of chemo- and radiotherapy, bone marrow transplantation and general immunostimulation. Although some cytokines are now in clinical use, it should be remembered that current knowledge is still limited and that the *in vivo* modulation of the activity of one factor may not have the desired effect. Knowledge of the biological mechanisms governing cytokine actions is an important contribution to the medical therapy of autoimmune diseases.

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