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Cytokine Secretion Patterns and Cross-Regulation of T Cell Subsets

Tim R. Mosmann

Department of Immunology, University of Alberta, Edmonton, Alta., Canada

Introduction

The immune system responds to different infectious agents with a variety of effector mechanisms, each of which is most effective against a particular type of pathogen. For example, antibodies are effective against soluble toxins and extracellular bacteria, and cytotoxic T cells lyse virus-infected cells. The response pattern induced by a pathogen is distinctive and often appropriate, resulting in the elimination of the infection, but there are severe consequences if the incorrect effector functions are induced. Induction of specific effector function is often carried out by T cell subsets secreting defined patterns of cytokines. Since the functions of T cells are mainly determined by the cytokines that they secrete, the different T cell subsets have markedly different functions. Regulation of immune effector functions may thus be at least partly due to the cross-regulation of different T cell subsets.

Subsets of Mouse and Human T Cells Defined by Cytokine Synthesis Patterns

Amongst mouse helper T (TH) cell clones, two distinct types of clone were originally defined according to their cytokine synthesis patterns [1, 2]. TH1 but not TH2 cells produce interleukin-2 (IL-2), interferon-γ (IFN-γ) and leukotriene (LT), whereas TH2 but not TH1 cells express IL-4, IL-5, IL-6, IL-10 and an induced gene of unknown function, P600 [1–4]. Both types of clone secrete several other cytokines, such as IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF) and members of the macrophage inflammatory protein family. Cytotoxic T cells secrete a TH1-like cytokine pattern, although IL-2 synthesis is often low or absent [5].

Although many mouse T cell clones fit either the TH1 or TH2 pattern, a number of laboratories have now described in vitro mouse T cell clones with other cytokine secretion patterns [6–8]. These patterns include the 'TH0' pattern, in which both TH1- and TH2-specific cytokines are secreted, as well as other patterns intermediate between TH1 and TH2 phenotypes. These non-TH1, non-TH2 patterns are obtained most often when both stimulator and responder cells are derived from unimmunized mice [6]. Since mouse but not human T cell clones are often derived from vigorously immunized individuals, these observations may help to explain the apparent discrepancy between the clear-cut TH1 and TH2 patterns of mouse TH cells, and the more mixed patterns, including TH0, of human T cell clones [9]. Some hu-

man TH1- and TH2-like patterns have been reported [10–12], and more examples are being discovered. Thus the final pictures of TH diversity in mouse and human may be quite similar.

Normal mouse and human T cells produce large amounts of IL-2 but not other T cell cytokines when first stimulated [6, 13, 14] and then a few days later are able to produce cytokines such as IL-4 and IFN-γ on restimulation. Thus naive T cells, or those that have not been recently stimulated, probably produce only IL-2 when first stimulated, and then differentiate into T cells producing multiple cytokines. Immunization in vivo causes similar results [6], and so it is likely that IL-2-producing cells (THp) differentiate into TH0, TH1 and TH2 phenotypes in vivo, in response to antigen stimulation.

Functions of T Cell Subsets

TH2 clones can provide excellent B cell help, whereas TH1 cells help less efficiently, or not at all [reviewed in 15, 16]. In some instances, help can be obtained even from 'non-helper' TH1 cells by increasing IL-2 and decreasing IFN-γ in the culture [15]. Part of the reason for the poor help provided by many TH1 clones may be their ability to kill B cells, probably via IFN-γ and LT production. Il-4, produced only by TH2 cells, induces isotype switching to IgG1 and IgE production [15]. In contrast, IFN-γ (from TH1 cells) inhibits all of the effects of IL-4 on B cells, and induces switching to γ2a. As a result, TH2 cells provide strong help for IgE synthesis and secretion, while TH1 cells are inhibitory. These cytokine effects have been confirmed in vivo using recombinant cytokines and monoclonal antibodies (mAbs) [17].

Although cell-cell signals are important for the initial activation of B cells, the cytokines secreted by the TH cells appear to be a more important influence on subsequent proliferation and antibody secretion. TH1 or TH2 cells can both provide the essential cell-mediated signal for IgE production, provided that IL-4 is present and IFN-γ is absent [15]. A similar situation may exist in macrophages, since the activation of tissue culture-grown macrophages for cytotoxicity requires IFN-γ, and a cell-mediated signal that can be provided by either TH1 or TH2 cells [18].

TH2 cells enhance two other features of allergic responses in addition to the stimulation of IgE secretion by IL-4. TH2 cells produce IL-3, IL-4 and IL-10, which all synergize to promote proliferation of mast cell lines in vitro [19, 20]. IL-5 induces the proliferation and differentiation of eosinophils [21]. Since mast cells and eosinophils bear Fcε receptors and use IgE as an antigen receptor for mediator release, TH2 activation may increase not only the level of IgE synthesized, but also the number of cells that bind IgE and respond to antigen.

TH1 cells cause an antigen-specific local inflammatory reaction when injected into the footpads of naive mice [22]. This reaction involves IFN-γ, and is more similar to Jones-Mote delayed-type hypersensitivity (DTH) than tuberculin-type DTH [23]. The tuberculin reaction may be mediated by a combination of TH1 and TH2 cells, or some other cell type, alone or in combination with TH1 cells. Since this reaction recruits and activates cells such as granulocytes and macrophages at the site of infection, DTH can be very effective against local infections, especially those involving intracellular parasites. TH1 cells also mediate several other cyto-

toxic functions that would be effective against intracellular pathogens [16]. Thus TH1 cells may be an effective response against intracellular organisms, and TH2 cells may be more important for producing antibodies to deal with extracellular organisms and their secreted products.

Cross-Regulation of TH1 and TH2 Cells

During many strong immune responses, antibody production and DTH are mutually exclusive. Since TH2 and TH1 cells can mediate antibody and DTH responses, respectively, the reciprocal relationship between these two responses could be explained by mutual inhibition of TH1 and TH2 cells. Two cytokines have such effects: IFN-γ inhibits the proliferation of TH2 cells, and IL-4 preferentially stimulates growth of TH2 cells [reviewed in 16]. Since DTH responses are often inhibited during strong antibody responses, we searched for a TH2-derived activity that would inhibit the growth and/or function of TH1 cells. We found an activity that suppressed the production of cytokines by TH1 cells responding to antigen + APCs [3]. The cytokine responsible for this activity, cytokine synthesis inhibitory factor, has now been characterized by purification and cDNA cloning.

Cytokine Synthesis Inhibitory Factor (CSIF, IL-10)

CSIF is an acid-labile homodimer of 35–40 kDa that is synthesized by TH2 but not TH1 cells or CTLs, and partially inhibits synthesis of most or all cytokines by TH1 but not TH2 cells. Cytokine production is not inhibited for the first 8 h after stimulation, but synthesis is inhibited very effectively at later times. Cytokines synthesized with more sustained kinetics, such as IFN-γ, are thus reduced to a greater extent than cytokines synthesized mainly at early times. In the presence of exogenous IL-2, CSIF does not affect the antigen-stimulated proliferation of TH1 clones [3].

Several experiments suggested that CSIF may inhibit TH1 cytokine synthesis indirectly. TH1 cells responding to antigen + APCs, or anti-T3 antibody + APCs, are inhibited, but stimulation by Con A, or anti-T3 bound to a plastic surface results in strong cytokine synthesis that is not inhibited by CSIF. Since its action requires the presence of APCs, we suggested that CSIF may act by inhibiting antigen presentation, or the production of a co-stimulatory signal required by the T cells [3].

cDNA Clones and mAbs for CSIF

cDNA cloning was carried out using the pcD-SRα cloning vector that drives high expression levels of functional protein in COS cells. After screening for activity in COS supernatants, a cDNA clone encoding CSIF activity was isolated [24]. The cDNA has an open reading frame of 178 amino acids, and the first 18 residues appear to be a hydrophobic leader sequence characteristic of a secreted protein. There are two potential N-linked carbohydrate attachment sites, and experiments using tunicamycin and glycosidase suggest that only some CSIF molecules are glycosylated [24], so that natural and recombinant CSIF each consist of polypeptides of 20 and 16 kDa. Since CSIF is a novel mouse gene with multiple biological activities in the immune system, we proposed that CSIF be named IL-10 [24]. Human IL-10 (hIL-10)

cDNA clones have been isolated [25], and recombinant hIL-10 inhibits cytokine synthesis by mouse and human T cells [25].

Six anti-mIL-10 mAbs were isolated after immunizing rats with partially purified mouse IL-10 and screening hybridomas using a solid-phase immunoadsorption technique [26]. Two antibodies were IgGs, that appeared to recognize partially denatured IL-10, since they did not block or adsorb IL-10 activity. The other four mAbs were all IgMs, and both blocked and adsorbed biological activity. Using two of the IgM mAbs that recognized different epitopes, a sensitive ELISA for IL-10 was established [26]. Since any of the four mAbs can deplete more than 95% of the CSIF activity from TH2 supernatants, IL-10 is the major or only CSIF produced by TH2 cells.

Since mouse $CD8^+$ clones synthesize the TH1 cytokine pattern [5], we tested the effect of IL-10 on cytolytic T lymphocyte (CTL) clones. As with TH1 clones, cytokine synthesis was inhibited but proliferation was not affected [T.A.T. Fong, D.F. Fiorentino, T.R. Mosmann, in preparation]. Thus IL-10 inhibits synthesis of the TH1 cytokine pattern by both of the cell types that express this pattern. As with many other cytokines, IL-10 is active on several cell types. These activities include stimulation of mast cell line [20] and thymocyte proliferation [27], and induction of Ia antigens and increased survival of small resting B cells [28].

Homology between IL-10 and an Epstein-Barr Virus Gene

A previously uncharacterized open reading frame in the Epstein-Barr virus, BCRF1, is highly homologous to mouse and human IL-10 throughout the mature protein coding sequence but not in the signal sequence or 5′- and 3′-untranslated regions. Recombinant BCRF1 protein inhibits IFN-γ synthesis by both mouse and human T cells [29]. Thus BCRF1 may represent a captured, processed mammalian IL-10 gene that has been conserved for the purpose of interfering with the host anti-viral response.

IL-10 in Parasite Infections

Mice infected with the helminth parasite *Nippostrongylus brasiliensis* mount a strong TH2-like response, since their spleen cells stimulated in vitro synthesize more IL-4 and IL-5, and less IFN-γ and IL-2 than controls [N.F. Street, T.R. Mosmann, unpubl.]. We have recently found that IL-10 synthesis is also greatly increased [T.R. Mosmann, unpubl.]. The high levels of IL-10 appear to be the cause of the decreased IFN-γ synthesis, since addition of anti-IL-10 mAb enhanced IFN-γ synthesis, even above control levels [T.R. Mosmann, unpubl.]. In another parasite model, mice infected with *Schistosoma mansoni* mount a strong TH2-like response after parasite egg-laying begins at about 4 weeks. By 7 weeks, cells from infected mice secreted substantial amounts of IL-10, but no detectable IFN-γ in response to antigen stimulation [A. Sher, T.R. Mosmann, submitted]. However, in the presence of anti-IL-10 mAb, significant levels of IFN-γ were synthesized in response to antigen. Thus IL-10 is a major cause of the suppression of in vitro IFN-γ synthesis by cells from parasite-infected mice. IL-10 may similarly suppress TH1-like functions in vivo during various parasite infections, thus enhancing TH2-like responses.

Conclusion

The cross-inhibition of TH1 and TH2 cells by IFN-γ and IL-10 may account for at least part of the mutually exclusive regulation of DTH and antibody (especially DTH) responses. It is likely that other cross-regulatory molecules exist, and we know very little about the regulation of the additional cytokine secretion phenotypes described above. In addition, the signals that induce preferential differentiation into a particular cytokine secretion phenotype are not yet understood, although it appears likely that this process is controlled by antigen-presenting cells and cytokines. Given the importance to the host of choosing the appropriate pattern of effector functions, it is likely that the full regulatory pathways of T cell differentiation and cross-regulation will be under precise and complex control.

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Tim R. Mosmann
Department of Immunology
Room 865 Medical Sciences Building
University of Alberta
Edmonton, Alta. T6G 2H7 (Canada)