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REVIEW

Cytokines: Communication Molecules that Influence the Process of Disease

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Abstract

Subsets of T lymphocytes that produce different cytokine patterns have reinforced the cellular- and humoral-mediated duality of the immune response. Thus, different cytokines selectively produced by different T cells results in the Th1/Th2 T cell paradigm. These T cell subsets, in an ever widening circle of examined diseases, contribute to the resolution or persistence of a disease. Now, it is timely to examine the contribution of the cytokines produced in different disease states for the improvement of vaccines and therapies for domestic animals.

Key words: cytokines, T cells, vaccine

The pathological consequences for a wide range of parasitic and infectious diseases are now linked to the pattern of cytokines produced by a particular T cell subset during the disease process. Presently, the connection between cytokine patterns and disease outcome is an intense area of interest in understanding disease mechanisms and prevention in domestic animals. The secreted cytokines play an important role in activating a variety of lymphoid cells, including, B cells, cytotoxic T cells, natural killer cells, macrophages and additional cells that participate in the immune response. Cytokine production by lymphoid cells occurs in distinct patterns allowing classification of cells into subpopulations. The most readily discernible cell populations are Th1 and Th2, also termed type 1 and type 2. These two cell populations have been best identified in CD4 T cells[4,21]. CD4 cells of the Th1 type produce interleukin (IL)-2, interferon (IFN)-γ and tumor-necrosis factor (TNF)-β, while Th2 cells produce IL-4, IL-5 and IL-6 and IL-13. Evidence has rapidly accumulated that Th1 and Th2 cells differ in the types of antibody response stimulated by B cells that Th1 cells mediate delayed type hypersensitivity reactions, and that Th2 cells are more potent helpers for antibody production, thus providing a simple explanation for the observed dichotomy in immune responses[44]. Two additional features of CD4 T cells are important in understanding the generation of helper-T cell diversity. First, each T cell subset produces cytokines that serve as autocrine growth factors and promote the differentiation of naive T cells to a given pathway. Second, the two T cell subsets produce cytokines that regulate the development and activity of the opposite subset in a paracrine manner. Thus,

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IFN-γ produced by Th1 cells amplifies Th1 development and inhibits proliferation of Th2 cells, while IL-10 and IL-4 produced by Th2 cells blocks activation of Th1 cells. This cytokine pattern evident in different T cell subsets supports the dual nature of T cell dependent effector and regulatory mechanisms, and the important roles played by cytokines in controlling pathological immune responses.

In the eleven years since the first description of helper T cell subsets, studying the roles of cytokine patterns is a major focus in understanding various diseases. Now, it is timely to propose hypotheses based on cytokine patterns to explain the differentiation and functions of T cell subsets and to explore these cytokine concepts as a basis for pathology, prophylactic vaccines and therapeutic approaches for immunologic diseases.

Function of Th1 and Th2 T cells

Controversy has existed regarding the primary functions for helper-T cell subsets. Th1 cells have been proposed as responsible for cell-mediated immunity and delayed type hypersensitivity, while Th2 cells were responsible for humoral immunity[4,44]. However, IFN-γ, considered as a Th1 cytokine, clearly is involved in determining the IgG isotype produced by B cells. Similarly, other Th1 and Th2 cytokines have biologic effects on humoral or cell-mediated immunity, respectively. Thus, the classification of cytokines into the two groups is based on an expanded understanding of function. Classification of cytokines within a particular subset is presently based on known biological activities. For example, IFN-γ activates macrophages, enhancing microbiocidal activity, as well as stimulating the production of IgG antibodies. Interestingly, IgG antibodies are central in opsonization and phagocytosis of particulate microbes by macrophages. In mice the IFN-γ induced isotypes are IgG2a and IgG3[13]. However, the IgG homologues induced by IFN-γ in domestic animals have yet to be clearly resolved[20]. Thus, a central function of Th1 cytokines appears to be a phagocyte-mediated defense against infections based on the ability of Th1 cytokines to activate macrophages to phagocytize and destroy microbes. In addition, Th1 cytokines, such as IFN-γ and IL-2 have autocrine and paracrine effects on natural killer and cytotoxic CD8 lymphocytes further substantiating a biologic role of Th1 cytokines in cell-mediated immunity.

In contrast, Th2 cytokines, e.g., IL-4 and IL-5, participate in the switch of B cell immunoglobulin production to IgE and eosinophil activation, respectively[24,61]. Cytokines produced by Th2 cells are known to help B cells produce IgM and non-complement-fixing IgG isotypes, IgG1, in mice but the effect of these cytokines on isotype production in domestic animals requires additional study[20].

Interestingly, several recently discovered cytokines appear to possess either cell-mediated or humoral immune enhancing abilities. The best characterized of these recent cytokines is IL-12 that is produced primarily by macrophages and is known to bias the immune response to a Th1 profile in contrast to IL-4 that directs a Th2 T cell response. A second cytokine, IL-14, also known as high molecular weight B cell growth factor, is produced by B cells and induces the rapid proliferation of B cell[22]. High concentrations of IL-14 has been observed in certain B cell tumors[22]. Similarly, IL-15 is expressed in skeletal muscle and serves as an anabolic agent to increase skeletal muscle mass by increasing myosin heavy chain expression[49]. Muscle cell division or the rate of myoblast differentiation appear unaffected by IL-15. IL-16 is produced by CD8 T cells and binds to the CD4 receptor present on CD4 T cells. Therefore, this cytokine may function in feedback communication between CD8 and CD4 cells. IL-16 is chemo-

tactic for CD4 T cells and induces functional IL-2 receptors on CD4 T cells[15,32,35,37]. Interestingly, IL-16 induces transmembrane signaling events similar to those noted for gp120 of human immunodeficiency virus (HIV) when it engages the CD4 ligand[7,8,39]. Perhaps one of the functions of the gp120 of HIV is to mimic a normal cytokine, like IL-16, to induce CD4 T cell activation. Another T cell cytokine that functions in T cell activation is IL-17. Northern analysis of RNA from various cells revealed IL-17 is produced in a tightly controlled manner by CD4 T cells and in stimulated peripheral blood T cells but not B cells or unstimulated T cells[23]. However, the receptor for IL-17 is produced by a wide variety cell types indicating that IL-17 acts in a proinflammatory manner on many other cells and tissues. IL-17 activates transcription factor NF-kB and induces expression of IL-6, IL-8, granulocyte colony stimulating factor, prostaglandin E2 and surface ICAM-1 and enhances proliferation of T cells induced by a sub-optimal costimulus, phytohemagglutinin (PHA)[63]. These findings suggest that IL-17 may constitute an early initiator of the T cell dependent inflammatory reaction and an element of the cytokine network that bridges the immune system to hematopoiesis. As with IL-16, IL-17 has an infectious mimic in Herpesvirus *saimiri*, a lymphotropic virus where the open reading frame 13 of the virus contains a sequence that has biologic activity with the IL-17 receptor[64]. Lastly, IL-18, also known as interferon-gamma inducing factor, augments natural killer cell activity similar to the structurally unrelated IL-12[43]. IL-18 enhances the production of IFN-γ and GM-CSF, while inhibiting IL-10 production in mitogen-stimulated lymphocytes[60]. IL-18 differs from IL-12 because IL-18 significantly enhances IL-2 and GM-CSF production in T cell cultures, while IL-18 and IL-12 similarly induce IFN-γ production by T cells. These findings suggest IL-18 enhances T cell proliferation through an IL-2 dependent pathway augmenting Th1 cytokine production[43] and cytotoxicity[16].

These newly identified cytokines have received minimal examination in domestic animals. The opportunity to explore the physiologic role of these cytokines in alternative species, as well as changes that occur in response to disease provides many avenues that may lead to the understanding of disease pathogenesis as well as new innovative vaccines and therapies regarding diseases of domestic animals.

T cell subsets in disease

Polarization of Th1 and Th2 T cell subsets can provide insight into immunological mechanisms of disease outcome. A number of infectious, allergic and autoimmune disorders have been associated with Th1 or Th2 cytokine profiles, suggesting the resolution or progression of given diseases pivot on the particular T cell subsets and their elaborated cytokines[31,51,52,56]. Again, the association of a cytokine pattern with a particular T cell subset support the concept of cell- and humoral-mediated immunity orchestrated by different T cell subsets. One of the first examples of this T cell subset duality associated with resistance or susceptibility to an infectious disease were inbred mice studies with cutaneous leishmaniasis caused by *Leishmania major*[51]. Impressively, cytokines produced by the Th1 subset (principally IFN-γ) in one mouse strain were associated with clearance of the parasite followed by disease resistance, while a different mouse strain produced cytokines associated with the Th2 subset (principally IL-4) resulting in disease persistence followed by death. The paradigm of cytokine profiles predicting the disease outcome has been reinforced using total tissue RNA obtained during the disease process and observing the Th1/Th2 cytokine patterns without the biases introduced by cell culture and cloning[54,62].

Resistance to many intracellular microbes,

including bacteria, protozoa and fungi, is founded on the induction of Th1 responses initiated by IFN-γ and TNF-α that activate macrophages[31,56]. The Th1 cytokine pattern also is evident in viral infections where natural killer cells, cytolytic CD8 T cells and neutralizing antibodies (usually with the Th1 isotype pattern) play the major role in host resistance[3]. In these Th1 responses, IL-12 produced by macrophages plays a pivotal role in directing the Th1 response pattern. However, the triggering events that predict IL-12 induction and the consequences of IL-12 to regulate the Th1 cytokine pattern is an active area of research. Relevant to predicting the Th1/Th2 cytokine pattern is the fact that IL-12 directly triggers the induction of Stat 4 supporting the Th1 cytokine pathway[26], while IL-4 triggers the induction of Stat6 and insulin-response substrate signaling Th2 cytokine pathway in T cells[25,36,53,55]. Supporting the role of these intracellular signaling pathways in development of Th1/Th2 cytokine patterns are the findings that deletion of the IL-12[41] or Stat4[30,59] gene result in deficient Th1 responses, while deletion of the IL-4[33,34] or Stat6 gene[29,57,58] result in deficient Th2 responses.

Relevant to Th1/Th2 cytokine patterns, progression of human leprosy to tuberculoid or lepromatous lesions are explained by the predominance of Th1 and Th2 cytokines, respectively[62]. Also, recombinant IFN-γ has been used to successfully treat the lesions of lepromatous leprosy supporting clinical cytokine application to influence disease progression in humans[28,45,46]. Similarly, progression of acquired immunodeficiency syndrome is predicted based on an early loss of IL-2, IFN-γ and IL-12 production[9,10]. Further, human immunodeficiency virus-1 appears to preferentially infect Th2 T cells, suggesting a mechanism for viral persistence in a Th1-deficient state[40].

A number of studies have examined the role of individual cytokines produced in response to pathogens of domestic animals; however, few studies have detailed the relationship of Th1/Th2 cytokine profiles in such diseases. Recently, studies of bovine leukemia virus (BLV) have determined that as disease progresses from the serologically positive to persistent lymphocytosis stage a shift in cytokine profiles of Th1 to Th2 occurs[48]. In the serologically positive stage IL-2, IFN-γ, and IL-12 are prominent cytokines. However, with progression to persistent lymphocytosis and tumor formation, these Th1 type cytokines decrease, while IL-10 becomes a prominent regulatory cytokine. The IL-10 present in this disease is produced by monocyte/macrophages and possesses regulatory properties that may be influential in disease progression. IL-10, originally termed cytokine synthesis inhibitiory factor[21], inhibits the expression of other cytokines especially Th1 cytokines, as well as reducing the expression of major histocompatibility complex class II molecules, mainly on macrophages, to inhibit antigen presentation[17]. The inability of T cells to respond to presented foreign peptides could reduce IL-2, IL-6 and IFN γ production. Second IL-10 also induces programmed cell death in Th1 cells[11,12]. Th1 cells express high levels of Fas ligand and can be induced to programmed cell death, whereas Th2 cells express low levels of Fas and fail to undergo apoptosis[50]. Fas antigen mediates Th1 cell activation early in the immune response as well as apoptosis of Th1 cells late in the immune response[1], and only activated T cells are killed by Fas-Fas ligand interaction[27,38]. Therefore, early in BLV infection Th1 cells may be activated, and later these activated Th1 cells may undergo programmed cell death. Thus, IL-2 and IFN-γ production might be reduced following apoptosis of Th1 cells. Such an imbalance between Th1 and Th2 cells may provoke the next disease stage of BLV infection as suggested for AIDS[12]. Exploration of Th1/Th2 cytokine patterns in diseases of domestic animals provides the oppor-

tunity to discern the mechanisms that underlie the basis of a given disease. A variety of reagents and approaches now exist to identify cytokines of domestic animals.

Identification of cytokines

Cytokines are most biologically active at short range and are often confined to the radius of several cells or in the intercellular space between two engaging cells, e.g., an antigen presenting cell and a T cell. Further, cytokines usually have an extremely short half life, with biological activity lasting only a few minutes in the blood prior to enzymatic inactivation. Further, cytokines that characteristically exist as membrane bound, e.g., IL-1 α or have already bound a receptor will be problematic in detection[18]. Thus, when selecting an assay for measuring cytokines, a particular assay may not be applicable for all circumstances. Although measurement of cytokine production in mice and humans has been aided by commercial enzyme-linked immunoabsorbent assays (ELISA), the general absence of species-specific commercial products has hampered cytokine analysis in domestic animals. In addition to ELISA methodology, bioassays, RT-PCR and flow cytometry can be used to assess cytokine production.

First, ELISA techniques are frequently used for cytokine assessment, this assay can only measure free, secreted cytokine. Further, monoclonal antibodies produced to murine or human cytokines most often detect a species-specific cytokine epitope, making such ELISA reagents useless for detection of domestic animal cytokines. Monoclonal antibodies to domestic animal cytokines are now receiving considerable attention. However, measuring cytokines in serum as an assessment of cytokine patterns suffers from the fact that cytokines principally act at short range. Similarly, measurement of cytokines from long-term cell cultures where oligoclones expand may not reflect the predominant cytokine pattern or cell population that existed in the animal. A modification of the ELISA is the ELISA-spot assay where cytokine production by single cells is detected[42]. This approach may allow cytokine assessment directly from an animal.

Second, bioassays utilize a cell line possessing the cytokine receptor. However, cells may possess receptors for additional cytokines that may be present in the sample to be tested. The use of cytokine specific monoclonal antibodies that compete for the cytokine, as well as recombinant cytokine as a standard are essential assay components. Presently, the monoclonal antibodies or recombinant cytokine are unavailable for most domestic animal cytokines limiting the utility of this assay system.

Third, reverse transcriptase-polymerase chain reaction (RT-PCR) assays can be used to measure cytokine transcription. This technique offers sensitivity, rapid assessment, small amount of cells are required and many cytokines can be simultaneously analyzed. RT-PCR allows the evaluation of immune responses occurring either systemically or within a local immune environment. Reagents to detect multiple domestic animal cytokines are often unavailable ; however, RT-PCR requires only the short segments of the DNA sequence from the respective cytokine gene[14]. Further, consensus sequence regions of similar cytokine genes of other species, such as mouse and human, often can be used to design appropriate primers. Using internal competitive standards (competitors) the absolute amounts of mRNA transcripts can be determined in small numbers of cells. Although cytokine transcription is assessed and not synthesis, reports document the strong relationship between transcription levels and amount of cytokine synthesized[2,5]. Similarly, oligonucleotide fragments may be used in Northern blot analysis[6] for the presence of cytokines.

Fourth, flow cytometry assays can detect

cytokine synthesis in the cytosol using cytokine specific monoclonal antibodies following cell membrane permeabilization[19,47]. This approach offers rapid, measurable detection of cytokines. Cell phenotype can also be determined; however, the number of fluorchromes that can be used simultaneously limits the number of cytokines detected in a given sample.

Conclusions

Identifying Th1/Th2 cytokine patterns produced by domestic animals during different stages of a disease provides insight into the mechanisms that underlie the basis of resistance or susceptibility. Thus, the scientific community is now positioned to test hypotheses regarding mechanisms of disease progression or resolution. With such knowledge comes the opportunity to influence the type and level of immunity through newly engineered vaccines that can preference a Th1 or Th2 cytokine pattern. Also, as concerns increase regarding antibiotic resistant bacteria, alternative methods to antibiotics are necessary to augment the host's own immune system to combat infectious agents. Knowing the role of Th1 and Th2 cytokines in particular diseases, it will be possible using chemical and biologic materials to enhance host immune responses by directing and augmenting a desirable Th1 or Th2 cytokine response. Lastly, because selected cytokines are instrumental in pivoting the development of a Th1 or Th2 cytokine response, certain cytokines or the genes encoding a given cytokine may be useful in domestic animal vaccines. Veterinarians and immunologists have a remarkable opportunity to creatively explore new solutions to long-standing diseases of domestic animals using the knowledge of individual cytokines and Th1/Th2 cytokine patterns.

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