Cytokines in giant-cell arteritis

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Giant-cell arteritis (GCA) in its typical form is a granulomatous disease that, for reasons yet unknown, targets the wall of medium-size and large arteries. Inflammatory infiltrates, composed primarily of T lymphocytes and macrophages, accumulate in the vascular wall. The tissue injury initiated by these inflammatory cells leads to the clinical manifestations of arteritis—most often ischemia and necrosis caused by vascular occlusion. In almost all cases, the arteritic lesions are combined with an intense syndrome of systemic inflammation. A definitive assignment for the site of the systemic inflammation has not been made. The existence of a related syndrome of systemic inflammatory activity in the absence of frank vasculitis, clinically identified as polymyalgia rheumatica (PMR), strongly suggests that GCA and PMR have an extravascular site of disease where inflammatory reactions unfold, leading to malaise, anemia, weight loss, night sweats, highly elevated acute-phase responses, and myalgias.

Cells in inflammatory infiltrates communicate by releasing hormone-like mediators, often categorized under the heading of cytokines. Resident cells in the tissue attacked by inflammation respond with the production of mediators, some of which are also classified as cytokines. Cytokines are directly involved in accomplishing the primary goal of inflammation, to eliminate the stimulus of tissue injury, cordon off the lesion to prevent spreading, remove tissue debris, and remodel the lesion with newly formed cellular and matrix components. Considering the complexity of inflammatory reactions, the need to orchestrate the interaction between an array of cells, and the diversity of tasks, it is no surprise that the list of cytokines recognized and implicated in inflammatory diseases is ever growing. Most knowledge is of interleukin (IL)-1, tumor necrosis factor (TNF)-α and IL-6. Some of the cytokines on the list have been studied in detail in GCA and PMR **(Table 1)**. These studies have provided valuable insights, and there is evidence that cytokines could be potentially helpful in the diagnosis and management of these diseases.

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TISSUE CYTOKINES IN GCA AND PMR—CLUES TO PATHOGENIC EVENTS

Macrophages are a major component of the cellular infiltrates in arteritic lesions, and they produce an array of cytokines, chemokines, growth factors, enzymes, and oxygen radicals. The most interesting aspects of macrophage biology in GCA have derived from the observation that a close correlation exists between the locale and the functional commitment of macrophages.[1] Vessel wall macrophages synthesize IL-1 and IL-6, often in conjunction.[2] IL-1β and IL-6 are released by macrophages in the adventitia; they rarely are produced in macrophages in the media or the intima. In the adventitia, IL-1/IL-6–producing macrophages partner with CD4 T cells that have all the features of recently stimulated lymphocytes.[3] T cells in the vascular infiltrate have undergone clonal expansion, suggesting antigen-driven responses.[4,5] The precise role of IL-1β and IL-6 in the granulomatous reaction is not entirely understood. Perivascular inflammation in mice with a genetic defect in the IL-1R antagonist gene suggests a direct amplifying contribution of IL-1 to vascular inflammation.[6] In GCA, a contribution of IL-1/IL-6–releasing macrophages in antigen presentation and T-cell activation is more likely. GCA is an HLA class II–associated disease, which alludes to a selective binding and presentation of arteritic antigens.[7] All available data indicate that this key event in the disease process occurs in the adventitia of the affected blood vessel, the site where IL-1/IL-6–producing macrophages accumulate.[8,9] Questions that need to be answered relate to the signals that adventitial macrophages receive in their microenvironment, the nature of the antigens they present on their surface, and the molecular interactions between adventitial macrophages and T cells.

Induction of IL-1β and IL-6 appears to be an early event in the disease process. This can be deducted from studies that identified IL-1β and IL-6 transcripts in temporal artery specimens from patients with PMR, arteries that lacked microscopically detectable infiltrates and were classified as negative for GCA by a pathologist.[10] Critical progress in understanding the pathogenesis of PMR and GCA could come from unraveling whether macrophages are triggered to produce IL-1 and IL-6 after they have entered the vessel wall through vasa vasorum, or whether they respond to a signal delivered in the periphery and then infiltrate into the arterial wall as a consequence.

Tissue production of IL-1 and IL-6 is highly sensitive to corticosteroid therapy. This has been useful in dissecting

TABLE 1
TISSUE CYTOKINES IN GCA

Cytokine	Possible function	Consequence/ outcome
IL-1β	Endothelial cell activation	Adhesiveness and cell recruitment
	Smooth muscle cell activation	Phenotypic/ functional switch
	Dendritic cell activation/ T-cell co-stimulation	Increased T-cell reactivity
TGF-β	Chemotaxis	Cell recruitment
	Fibroblast activation	Intimal hyperplasia
	Matrix synthesis	Intimal hyperplasia
IL-6	?	?
IFN-γ	Macrophage activation	
	–monokines and chemokines	Amplification of inflammation
	–metalloproteinases	Digestion of elastic membranes
	–oxygen radicals	Smooth muscle cell injury
	–growth factors	Intimal hyperplasia
	–angiogenesis factors	Neoangiogenesis

the anti-inflammatory activities of dexamethasone and acetyl salicylic acid in GCA.[11,12] Interestingly, the functional activity of adventitial macrophages cannot be abrogated by corticosteroid treatment. Macrophages in the adventitia persist and continue to synthesize TGF-β. The contribution of this cytokine to GCA is unresolved, but the chemotactic activities of TGF-β may contribute to the chronicity of the disease. TGF-β could also have a central role in the events leading to the mobilization, migration, and hyperproliferation of myofibroblasts and the deposition of matrix in the formation of lumen-occlusive intimal hyperplasia.

A key cytokine in the arterial wall is interferon (IFN)-γ.[3] It derives from CD4 T cells in the adventitia of affected blood vessels. It is an indicator of an adaptive immune response, reemphasizing the roles of antigen recognition and T-cell responsiveness as critical pathogenic steps. In model systems of GCA, depletion of IFN-γ–producing T cells is the only predictable intervention to abrogate vasculitis.[13] IFN-γ is the most potent activator of macrophages, and it almost certainly functions in the arterial wall by orchestrating the differentiation of macrophages into effector cells with tissue-destructive potential. Macrophages in the media become a source of oxidative stress with lipid peroxidation products attacking smooth muscle cells.[14-16] Distinct macrophage populations produce growth factors, such as platelet-derived growth factor (PDGF), and angiogenesis factors, such as vascular endothelial growth factor (VEGF).[17,18] These growth and angiogenesis factors are instrumental in driving intimal hyperplasia, the ultimate process leading to vaso-occlusion and ischemia. Tissue concentrations of IFN-γ and growth factor transcripts vary considerably between different patients, but they correlate with each other and the degree of intimal hyperplasia. This variability is biologically relevant, as emphasized by the correlation between clinical phenotype of GCA and the tissue concentrations of key cytokine transcripts, in particular IFN-γ **(Table 2)**. The central role of IFN-γ in these events makes this cytokine a preferred target for therapy as well as for distinguishing different types of vascular reactions.[19]

CIRCULATING CYTOKINES IN GCA AND PMR—TOOLS FOR DISSECTING DISEASE HETEROGENEITY

Circulating cytokines in GCA and PMR attract attention for two reasons. They are easily accessible, making them preferred tools in clinical practice, and they provide information about the systemic component of GCA and PMR, a component that is, at least in part, independent of the vascular component. Acute-phase reactants and downstream consequences of acute-phase responses have long been used as diagnostic clues for GCA/PMR, usually by measuring erythrocyte sedimentation rates (ESR) and C-reactive protein (CRP) levels. Acute-phase proteins are released upon cytokine signaling, mostly from hepatocytes. They are believed to play a role in tissue defense, tissue repair, and, when produced excessively, tissue damage. A critical cytokine in acute-phase induction is IL-6, formerly known as hepatocyte stimulating factor. IL-6 has wide-ranging biological activities, crudely divided into those regulating the hematopoietic system and those modulating innate immunity.[20] IL-6 stimulates B lymphocytes and drives proliferation of hematopoietic and megakaryocytic progenitors. Its action on non-immune cells is tied to activation of the hypothalamic-pituitary-adrenal axis. It is pyrogenic and is the most potent inducer of hepatic acute-phase proteins. Possibly important in the context of GCA and PMR is the ability of IL-6 to downregulate monokine production, such as TNF-α and IL-1β, and to suppress chemokine secretion. In essence, it may be regarded as a physiologic anti-inflammatory cytokine with potentially beneficial effects.

More than any other cytokine, IL-6 functions like a hormone and is released into the circulation.[21] Its cellular source in GCA and PMR is unknown. It could be speculated that it derives from vasculitic infiltrates, yet IL-6 levels are highly elevated in patients with PMR who have minimal arterial wall lesions, suggesting an alternative source. Given the marked changes in bone marrow function in PMR/GCA, this primary lymphoid organ is a prime suspect as the site of the intense systemic inflammatory reaction. Patients with GCA/PMR often have signs of suppressed red blood cell production combined with accelerated turnover of megakaryocytes. The precise mechanisms are unclear, but a recent study by Orphanos et al[22] has described a switch in the cytokine gene expression profile of marrow stromal cells of patients with GCA. Specifically, marrow samples of GCA patients expressed reduced amounts of stem cell factor, TGF-β, and TNF-α

and instead contained IL-1α and IFN-γ.

Circulating macrophages in patients with untreated GCA/PMR are constitutively activated, and 60% to 80% of them produce IL-1β and IL-6.[2] This intense stimulation of the innate immune system could result from a number of stimuli. Compounds triggering pattern recognition receptors as microbial products, stress proteins, or bacterial or viral DNA/RNA, could all rapidly induce macrophage activation. Alternatively, a powerful activator of monocytes/macrophages is IFN-γ, a specific product of the immune system responding to antigenic challenge. While inflammation of the arterial wall is T-cell dependent, it is not known whether circulating cytokines in GCA are a reflection of an innate immune response or are the result of T-cell recognition of a defined antigen.

IL-6 is highly elevated in patients with GCA and PMR.[23] Serum levels rapidly respond to immunosuppression with steroids and closely correlate to clinical symptoms, particularly those of myalgias and stiffness. The high correlation between IL-6 and clinical presentation has encouraged studies using the marker to dissect the clinical heterogeneity of PMR and GCA **(Table 3)**. In a prospective study of patients with PMR, IL-6 plasma concentrations were helpful in distinguishing patients in terms of steroid requirements and prognosis.[23] Pretreatment IL-6 values of <10 pg/ml were found in patients with a benign disease course characterized by lack of disease recurrence and steroid treatment <1 year. If pretreatment levels of IL-6 in the circulation were >10 pg/ml, patients fell into two categories. Those with normalization of IL-6 concentrations upon initiation of steroid therapy required one to two years of treatment and had infrequent clinical flares during tapering. Patients with high pretreatment IL-6 levels and continuous production of IL-6 despite corticosteroid therapy were classified as non-responders. They required increasing doses of prednisone to control clinical symptoms, had frequent exacerbations of clinical symptoms with dose reduction, and included patients who progressed to full-blown vasculitis. Studies in larger patient cohorts are necessary to understand the potential of IL-6 in differentiating clinically meaningful subsets of PMR. As we strive for patient-targeted therapy, blood cytokines are the most promising tools in assigning patients to diagnostic and therapeutic categories.

TABLE 2
TEMPORAL ARTERY CYTOKINE PATTERNS AND DISEASE HETEROGENEITY

Disease phenotype	IL-2	IFN-γ	IL-1β	PDGF	VEGF
PMR	++*	–	+	–	?
Aortic arch syndrome	++	+	+	?	?
Fever and wasting	++	+	+	–	–
Jaw claudication, visual loss	+	+++	+++	+++	+++

* –, transcripts not detected by PCR;
+ - +++, transcripts present at different levels.

CIRCULATING CYTOKINES IN GCA AND PMR—BIOLOGICAL MARKERS OF DISEASE ACTIVITY?

The important practical issue of classifying GCA and PMR patients into subsets with distinct therapeutic needs is closely related to monitoring disease activity during immunosuppression. Empirically, corticosteroids have been superior to any other immunosuppressive drug in treating this inflammatory vasculopathy. Mechanistic studies in vivo have indicated that corticosteroids target the NF-κB pathway and the genes dependent on this transcription factor. These studies also revealed that corticosteroid therapy only inhibits some mediators while others are relatively resistant.[12] In fact, vascular infiltrates persist in an animal model of GCA as well as in patients despite ongoing corticosteroid therapy. These findings have suggested an interesting discrepancy between the success in improving clinical symptoms and the failure of eliminating vascular lesions. This discrepancy provides an explanation for the chronicity of the disease with a need for prolonged immunosuppression although patients improve dramatically within a few days. Impressive clinical improvement despite persistent vasculitis also re-emphasizes that the disease has two components. These two components may vary profoundly in their sensitivity towards the immunosuppressive effects of corticosteroids.

IL-6 is rapidly responsive to corticosteroids, in holding with the role of NF-κB in the regulation of the IL-6 gene. The upstream positioning of IL-6 in the cascade of acute-phase responses would encourage use of this marker to carefully monitor activity of the disease process. Available data suggest that IL-6 might be an ideal candidate to titrate steroid requirements. In a cohort of patients with biopsy-proven GCA, serum IL-6 levels were superior in detecting vasculitis when compared with traditional laboratory parameters.[25] IL-6 was above normal in 92% of all untreated patients, whereas ESR identified only 74% of the patients. All patients were treated with glucocorticoids using a predetermined protocol of steroid tapering and all responded to initiation of therapy with normalization of the ESR. This excellent response rate did not apply to concentrations of circulating IL-6. In only 46% of the patients could 60 mg prednisone per day suppress IL-6 into the normal range. Upon follow-up of the patients, ESR results were only partially helpful in making decisions whether the patient was sufficiently treated or suffered from a disease flare. IL-6 detected almost 90% of disease reactivation. More importantly, IL-6 remained moderately elevated in patients considered clinically to be non-active or in remission. IL-6 outperformed CRP and ESR in demonstrating ongoing disease activity. Indeed, the marker seems to be so sensitive that a new therapeutic issue in managing GCA has arisen. Should the patient be treated until all clinical signs of inflammation are controlled, or should we attempt to reach total suppression of the disease process, including the release of pro-inflammatory cytokines? What is the risk of a patient experiencing clinically undetectable but biochemically detectable

TABLE 3
PERIPHERAL BLOOD IL-6 LEVELS AND DISEASE SEVERITY

Disease severity	Pre-treatment	Treatment	Flares	Post-treatment
Mild PMR	<10 pg/ml	Normal	No flares	?
Moderate PMR	>10 pg/ml	Normal	Increase in IL-6	?
Resistant PMR	>10 pg/ml	>10 pg/ml	Increase in IL-6	?
GCA	>10 pg/ml	>10 pg/ml	Increase in IL-6	Elevated

disease? What is the site of disease? Results in the animal model of GCA would suggest that vascular infiltrates persist. Are they the source of IL-6 and do they pose a risk to the patient? Does the risk of side effects from more aggressive therapy outweigh the benefit for the patient? Appropriately designed therapeutic trials will be able to give answers to some of these questions, but the community of physicians must be prepared to follow patients for more than a decade because late disease manifestations may not be detectable prior to that time.

SUMMARY

Cytokines are small proteins that serve as chemical messengers between cells, regulating cell growth and differentiation, tissue repair and remodeling, and many aspects of the immune response. Cytokines are instrumental in determining the nature, magnitude, and duration of inflammatory reactions and, as such, represent ideal targets for interfering with pathogenic processes. In GCA and PMR, cytokines are encountered in two locations, the inflammatory infiltrates accumulating in the arterial wall and in the circulation. IL-6, a cytokine involved in stimulating acute-phase responses, is located upstream of many of the laboratory abnormalities considered helpful in diagnosing and managing GCA/PMR, including elevated ESR and CRP. IL-6 has the potential to be helpful in predicting disease severity and may allow for a tailoring of immunosuppressive therapy. There is evidence suggesting that IL-6 outperforms other chemical markers in detecting disease activity and could, therefore, have a role in monitoring treatment. Interesting pathogenic clues have been derived from studies of cytokines produced in the vascular lesions. IFN-γ has emerged as a key regulator in determining the nature and direction of the inflammatory response. IFN-γ appears to be critically involved in modulating the process of intimal hyperplasia, the most destructive consequence of vasculitis, and, as such, emerges as a prime target for novel therapeutic approaches.

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