

Cytokine polymorphisms and inflammation

P. Woo

Professor Patricia Woo, Centre of Paediatric and Adolescent Rheumatology, The Windeyer Institute of Medical Sciences, Department of Molecular Pathology, University College London, 46 Cleveland Street, London W1T 4JF, UK. *Clin Exp Rheumatol* 2000; 18: 767-771.

Received on August 2, 2000; accepted on August 31, 2000.

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Keywords: Cytokines, chemokines, polymorphism, inflammation.

ABSTRACT

Cytokines and chemokines are important mediators of inflammation. The numbers are still expanding. It is clear that these mediators act in positive and negative feedback loops in a complex network to allow inflammation as well as repair to take place. The fact that many of these molecules and their receptors are polymorphic with functional consequences is becoming clear. Such genetic polymorphisms will impact on the network to produce a variety of inflammatory responses, both acute and chronic.

Introduction

Inflammation is essential to protect the integrity of the individual against physical, chemical and infective insults, and to initiate the process of repair. A large number of physiological changes occur during the process of inflammation. There is recruitment of cells and soluble mediators in response to the external stimulus. The effect is the elimination of the pathogen and /or the injury, followed by repair of the affected tissues. These changes have positive and negative feedback mechanisms so that resolution is generally achieved. Inflammation can cause disease when it becomes chronic as a result of either the failure of the normal mechanisms for the resolution of inflammation or the persistence of the stimulus.

Acute versus chronic inflammation

Acute inflammation is a normal reaction to an external insult. There is a marked systemic acute phase response. The response can include rash, fevers, systemic disturbances and acute reactive arthritis. Histology of the lesion typically reveals the accumulation of leucocytes, predominantly neutrophils, and the exudation of fluid and plasma proteins. The processes are sequential and complex, initiated by vascular permeability, extravasation of inflammatory cells, ma-

trix deposition and tissue repair.

The lesion of chronic inflammation is typified by the infiltration of lymphocytes and monocyte/macrophages and by the activation and proliferation of connective tissue. It is thought to be either the result of persistence of an infectious agent, such as *Staphylococcus aureus* in osteomyelitis, or the failure of the regulation of inflammation due to genetic variations in the molecules that mediate the innate and adaptive immune response. Thus, the same agent can produce a spectrum of disease phenotypes depending on the genetic make-up of the individual. Furthermore, genetic polymorphisms can potentially influence the susceptibility of the individual as well as the pattern of inflammatory damage. Malignant clones of cells can also cause inflammation, such as Castleman's disease where cytokine-secreting tumours secrete predominantly interleukin-6, thereby causing multisystem inflammation.

The cytokine network

Cytokines are a group of potent polypeptide mediators secreted by a large variety of cell types to regulate cell growth, differentiation, and activation through specific receptors on target cells. Within the immune system, the cytokines produced by leukocytes mediate the immune response and inflammation, and are named interleukins. There are other cytokines that are also produced by leukocytes, and are functionally related to this group of interleukins, e.g. interferon (INF), tumour necrosis factor (TNF), and transforming growth factor (TGF). Traditionally interleukins are often divided into those that are largely responsible for immunoregulation, that is, antigen presentation, lymphocyte proliferation and differentiation (e.g., IL-2, 3, 4, 5, 9, 10, 12, 18, INF, TNF etc.), and those that are responsible for the inflammatory response (e.g.,

IL-1, 6, 8, 10, TNF). However, the functions of many of these molecules can overlap with each other, and are often effectors within both areas. The biologic activity of a given cytokine *in vivo* is a net effect determined by its concentration and by the presence of specific inhibitory molecules, such as IL-1 receptor antagonist (IL-1ra), soluble TNF receptors (sTNFR), and general inhibitors of pro-inflammatory cytokine synthesis e.g. IL-10. In other words, the balance of cytokines with opposing functions is an important factor in the initiation as well as the resolution of inflammation. The dynamics of cytokine production is non-linear *in vitro*. There is also *in vivo* evidence of a non-linear dynamic network, and the position of these opposing molecules within the network would also influence the final stable state of the network. Animal gene knockouts demonstrate non-linear gene dose effects, e.g. in TNF knockout mice (1). Certain cytokines such as TNF and to some extent IL-1 serve as amplifiers in murine and human inflammatory arthritis so that antagonists to these two cytokines can produce large changes within the network and resolution of inflammation (2).

Cytokine gene polymorphisms

The coding sequences of cytokine and cytokine receptor genes are generally well conserved. Mutations usually lead to alteration of the function of the protein and subsequent pathology. Cytokine genes are highly polymorphic in the 5' and to a lesser extent the 3' and intronic regions. A recent database was set up to catalogue the polymorphisms by Bidwell, Gallagher and colleagues (3). Studies of the polymorphic variants of cytokine and cytokine receptor genes have shown *in vitro* and *in vivo* variations in gene expression, e.g. TNF (4, 5), TNF (6), IL-1 gene cluster (7, 8), IL-6 (9, 10), and IL-10 (11, 12). Most of these variations are single nucleotide polymorphisms (SNP) with functional consequences and they may represent candidate alleles for disease susceptibility or severity, as well as responders and non-responders to drug therapies.

Cytokine and cytokine receptor genes as candidates for susceptibility to inflammatory and autoimmune diseases have

been studied by case control association studies in the majority of cases. Most of these will require replication in other populations, or in simplex families (i.e., parents and proband). Of interest is a meta-analysis by Becker *et al.* (13) of 23 genome-wide scans of murine and human inflammatory and autoimmune inflammatory diseases. Approximately 65% of the human positive linkage maps available at the time were in 18 non-random distinct clusters, many of which include loci for cytokine, chemokine and their receptors. In addition to these analyses, a cytokine cluster on chromosome 5q31 (which includes interferon regulatory factor IRF1, IL-5, 4, 3, 12 p40, 13) was found within the mapped region for asthma.

While these are tantalising data, association studies of candidate genes using the transmission disequilibrium test (TDT) is generally accepted to be more sensitive than genomic linkage analysis with microsatellites in identifying candidate susceptibility genes with relatively weak effects. A relative risk of 2 is commonly found for genes associated with diseases that are thought to be complex genetic traits. Furthermore, genetic association may be with disease severity, which represents a genetic reason for the clinical outcome of that particular disease. For example, patients with cerebral malaria and the -308 TNFA2 allele have an 8-fold increased risk of death (14).

Cytokine and T-cell polarisation

After exposure to antigen, precursor helper T lymphocytes differentiate into two major types of helper T cells with respect to their function and the range of cytokines they produce (15). The degree of polarisation and heterogeneity of T lymphocytes may reflect the nature of the antigenic and environmental stimuli to which the cells have been exposed. This is particularly true of responses to persistent infections with microbes such as *Leishmania*, *Listeria*, mycobacteria (Th1) and helminths (Th2), or to non-infectious persistent antigens as in allergies and autoimmune diseases, where Th2/1 polarisation respectively is frequently found. The cytokines produced by Th1 T cells are IL-2, 3, IFN, granulocyte-macrophage colony-stimulating

factor (GM-CSF), and TNF and Th2 cells secrete IL-3, 4, 5, 6, 10, GM-CSF, TNF, and IL-4. These two major pathways of T cell differentiation are mutually antagonistic. Two other "regulatory" or "anti-inflammatory" CD4+ T cell subtypes have been described: Th3 cells that are characterised by secretion of TGF, and Tr1 cells that are characterised by the secretion of IL-10.

Differentiation into these types of cells occurs mainly under the influence of two cytokines. IL-12 is produced by monocytic antigen presenting cells including dendritic cells. In conjunction with costimulatory molecules, IL-12 promotes the differentiation of potential T helper cells (Th0) to an IFN secreting Th1 phenotype, which is responsible for cell mediated immunity. Mutations in the coding region of the IL-12R 1 gene has been found in disseminated mycobacterial and salmonella infections (16). Mutation in the extracellular domain of the IFN R1 similarly lead to disseminated mycobacterial infections (17, 18).

IL-4 is the cytokine with autocrine function that promotes T cells to differentiate into the Th2 phenotype, responsible for T cell help in the antibody response. Polymorphic alleles of the IFN R1 and 2 as well as polymorphic alleles of the IL-4R chain have been described. IgE (19) and atopy (20, 21) have been associated with these polymorphisms and may suggest that the degree of T cell polarisation is influenced by these genetic variants. Further confirmation from other cohorts and family studies are needed. Predominantly Th1 type T cells are found in certain autoimmune diseases, e.g. insulin dependent diabetes (IDDM), rheumatoid arthritis (RA), multiple sclerosis (22-24) and oligoarticular juvenile idiopathic arthritis (JIA) (25), leading to the hypothesis that the prolonged imbalance of T helper subtypes may lead to pathology (15). In IDDM, a CA repeat polymorphism of the IFN gene was found to be associated with susceptibility (26), while the IL-1 cluster was not (27). A Japanese study suggests that a TNF microsatellite polymorphism was increased significantly in the young onset IDDM (28). There have been conflicting reports of case-control studies in different populations of RA and the possi-

ble association with different alleles of the TNF gene. The possibility of these finding being a function of linkage of the TNF gene to the real susceptibility gene within the MHC region is not resolved, and there is no TDT confirmation of any of these findings so far. Cantagrel *et al.* (29) showed in preliminary studies that a polymorphism in the IL-4 gene is associated with RA, and that alleles of IL-1 and may be associated with more erosive/severe arthritis.

Atopy and asthma are thought to be predominantly Th2-like diseases, and the mapping of asthma to the cytokine-rich region of chromosome 5q31-33 is suggestive that cytokines and their receptors involved in the polarisation of T cells may be important.

Cytokines and inflammation

Imbalances toward pro-inflammatory cytokines are induced by infectious agents, e.g. there is a major increase in the pro-inflammatory cytokines IL-1 and TNF but very little IL-1ra when PBMC are cultured with the Lyme agent *Borrelia burgdorferi* (30). In the case of autoimmune diseases, however, a persistent imbalance towards a proinflammatory state is found. This could mean that there is a persistent antigen present or else there is a genetically determined imbalance within the cytokine network so that the stable state is proinflammatory. There have been many studies of the anti-inflammatory gene IL-10 and the allelic associations with inflammatory rheumatic diseases. In SLE there has been a report of an association of a microsatellite (G.9) allele of IL-10 in a large case control study of Mexican Americans (31), and IL-10 SNP haplotypes (GCC or ACC) have been found to be associated with anti-Ro in one report, but not in another (32, 33). Similarly, an association of lupus nephritis with the ATA haplotype, which has been shown *in vitro* to be transcriptionally a low responder to LPS (11), was found in a small population of southern Chinese, but not in a Caucasian population (33, 34).

Although there have been many reports of raised levels of inflammatory cytokines in the synovial fluid and plasma of children with JIA, it has been difficult to demonstrate an imbalance. This

is mainly due to the instability of some inflammatory cytokines and the differing sensitivities of the methods of detection of the individual cytokines and their antagonists. Martini and colleagues (35) have been unable to show a difference in the ratio IL-1RA: IL-1 in the synovial fluids of different types of JIA. Work in this laboratory showed that the molar ratio, sTNFR:TNF, in the synovial fluids of polyarticular JIA was significantly reduced in comparison with a group of children with spondyloarthropathy (HLA B27 positive oligoarticular disease), suggesting that higher levels of TNF may be contributory to more severe erosive disease (36). We therefore tested the hypothesis that the expression of the general anti-inflammatory cytokine IL-10 is genetically lower in the more severe JIA subtype. The production of the general anti-inflammatory cytokine IL-10 was lower in the parents of children with extended oligoarticular JIA, and these parents had a significantly increased frequency of the ATA IL-10 haplotype (37). The children with the more severe disease (extended JIA) had a significantly increased frequency of the IL-10 ATA haplotype. TDT confirmed the disease association of the IL-10 ATA allele with this group of children. In addition, TDT showed that there is significant association of the ATA IL-10 allele with a clinical trait, uveitis, found in 3 subgroups of JIA (37).

In asthma, the ATA allele of IL-10 was also significantly raised in the more severe cases in a case control study (38), thus providing further evidence for the IL-10 ATA allele as a possible severity gene in inflammatory diseases. There are a large number of SNPs further upstream of these haplotypes, and 2 microsatellites. Therefore, a dense SNP map of the gene will be necessary to see which alleles are important in inflammatory diseases.

Of great interest are the dominant familial periodic syndromes, such as familial Hibernian fever, also known as TNF-receptor-associated-periodic-syndrome (TRAPS). There is a mutation in TNFR1 so that it is not easily cleaved into the soluble form. The result is low sTNFR1 levels on stimulation, and the subsequent imbalance between TNF:sTNFR1 leads

to inflammation (39).

As far as the IL1 locus is concerned, McDowell *et al.* (40) have shown that the IL-1A2 polymorphism in JIA is associated with a higher risk for developing uveitis in Norwegian oligoarticular JIA. But Donn *et al.* (41) failed to confirm this association in English JIA. This association will need confirmation by the transmission disequilibrium test in Norwegian families to see if the IL-1 locus has an influence on the phenotype of oligoarticular JIA.

The case for systemic JIA being an interleukin 6-mediated disease was well argued in a recent editorial by De Benedetti and Martini (42). Many of the clinical features are typical of excessive IL-6 production, e.g. fever, hypergammaglobulinaemia, thrombocytosis, anaemia and stunted growth. We have identified a functional polymorphism in the regulatory regions of the IL-6 gene that determines a transcriptional response of the IL-6 gene to IL-1 and LPS. There was a significant lack of the protective genotype (CC: low producer of IL-6 on stimulation by IL-1/LPS) in children that develop systemic JIA at age 5 and under (9). TDT of an association with IL-6 in simplex families are in progress to see if this can be confirmed. Further SNPs have been found and analyses of haplotypes suggest a more complex genetic regulation of IL-6 (10). IL-6 has been implicated in growth failure (42) and cardiovascular risk (43). Therefore, identification of functional SNP haplotypes and re-examination of these disease cohorts will be necessary.

The above studies represent a new perspective on the function of these effector molecules. More work in this area should yield a "map" of the potential points of deregulation within the cytokine network of the individual with a chronic inflammatory disease.

Other molecules related to the cytokine network

Chemokines have specificity of actions on leucocytes of particular lineages (44). The chemokine family has 40+ members. The first one described was IL-8. They are small proteins of 8-10 kD, with four conserved cysteines forming two essential disulphide bonds. There is re-

dundancy and non-linearity in the system, as in the cytokine network. The chemokines have important functions in the migration of lymphocytes, monocyte and neutrophils to sites of inflammation. They are released by leucocytes in response to cytokines such as IL-1 and TNF. Chemokines are heparin-binding proteins and can bind to surface glycosaminoglycans, thus acting as important molecules in the regulation of movement of leucocytes along a "bound chemo-attractant gradient". Chemokines are involved in homeostatic function in lymphoid tissues. The traffic of T and B lymphocytes through the different tissue compartments are regulated by the constitutive tissue expression of chemokines, and the maturation of B lymphocytes for example appear to depend at least in part the presence of specific chemokines such as stromal derived factor-1 (SDF-1).

In allergic inflammation, cells possessing CCR3 chemokine receptors are recruited, and they include basophils and eosinophils as well as Th2 T cells (found to be pathogenic in allergic inflammation). Recent research has shown that T lymphocytes express different chemokine receptors during differentiation and activation. Thus, Th1-like T cells would express preferentially CCR5 and the Th2-like T cells, CCR3 and CCR4. The implication is that they traffic differently and would have influence on the course of the local immune response. CCR5+ T cells (Th1-like phenotype), for example, accumulate selectively in the rheumatoid joint, compared to the peripheral blood (45). This could be a selective recruitment due to surface receptors on endothelial cells and/or the result of the chemokines present in the synovium and synovial fluid. In support of the latter mechanism, the CCR5 ligands RANTES and MIP-1 are found at high levels in the synovial fluid of RA.

Of much interest is the association of chemokine receptor mutation/polymorphisms in the protection/transmission of HIV infection. In the area of inflammatory diseases, a functional mutation in the promoter of the C-C chemokine RANTES has been found in association with atopic dermatitis (46). Functional polymorphisms of the chemokine recep-

tor CCR2 and CCR5 have been reported to be associated with IDDM in a case control study in Hungary, but will require confirmation.

Conclusion

The cytokine network is central to inflammation. Our understanding of the interactions within this non-linear, dynamic network *in vivo* is further hampered by genetic polymorphisms of the genes. Many of these polymorphic variants regulate gene expression and so have the potential to "set" the network into a stable state that is pro-inflammatory. Candidate gene association studies using the case-control method in some infectious and autoimmune diseases suggest that this hypothesis could be correct. So far, there is one confirmation of an association (using the TDT) of a candidate cytokine gene: the association of IL-10 and a JIA subgroup. Further studies of this type are needed to confirm the association and to eliminate any false association results due to population stratification in the case control studies. Identifying the disease phenotype modifying effects by polymorphic variants of the cytokine network will have clinical importance. Some of the benefits would include the prediction of disease outcome and the response to drug therapy.

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