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Cytokines in childhood rheumatic diseases

The term cytokines now embodies a large group of important polypeptides whose functions are extremely diverse but are responsible for growth and differentiation of cells, cell to cell signalling, which include mediating the immunological responses and inflammatory responses, and hormonal functions: endocrine, autocrine, and paracrine. The classification of these multifunctional proteins is still evolving. They can be either classified according to their functions, or to their structural similarities.

There are now 12 interleukins which are proteins or polypeptides that mediate cell to cell signalling of the leucocytes. These can be subdivided to the largely monocyte derived cytokines and lymphocyte derived cytokines. This division has some advantage in approximately defining two broad groups of function – that is, inflammatory and immune (see table 1).

Another broad group of cytokines are called growth factors, which are mainly involved in the differentiation and maturation of stem cells into the different lineages in bone marrow (see figure). One can see already some of the overlap, for example interleukin-1 (IL-1) and IL-6 also have differentiation and growth functions as well as being important in cell signalling. A third group of cytokines are the interferons, which have antiviral properties as well as cell regulatory and differentiation properties.

Finally, there is another group of proteins, which are important for matrix formation especially in fibrous tissue, bone, and cartilage – that is, the transforming growth factors and bone morphogenic proteins. This is by no means a comprehensive list but very roughly categorises them according to their main functions.

It is becoming clear that there are complex homoeostatic mechanisms within this cytokine network, for example IL-4 and IL-10 and the newly discovered IL-12 reduce synthesis of IL-1 and tumour necrosis factor-α (TNF-α) and could therefore be classified as anti-inflammatory cytokines, although they have other immunoregulatory roles in lymphocyte function. Other so called anti-inflammatory cytokines include transforming growth factor-β_1 (TGF-β_1). The inflammatory cytokines are IL-1, TNF-α, and IL-6. (Their functions are listed in table 2.) All the interleukins have soluble receptors that are thought to be shed from the cells rather than actively secreted. These soluble receptors act as inhibitors of the cytokines and thus modulate their biological activities. There is one exception, the IL-6 receptor acts as an agonist for IL-6, because it can still interact with the signalling protein on the cell membrane (gp130).

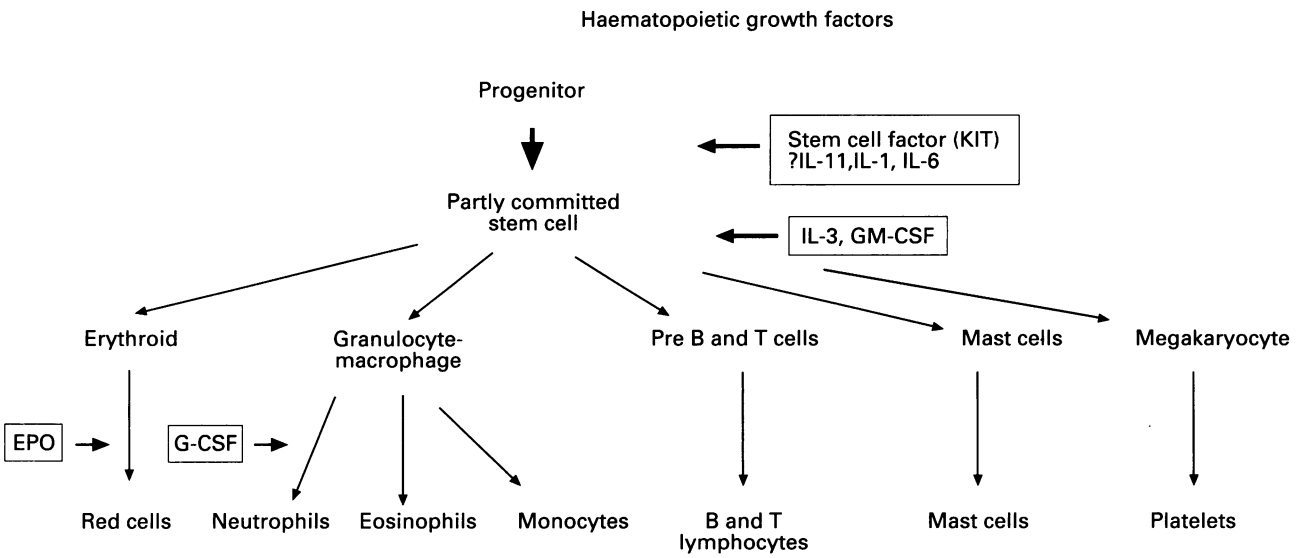
In acute inflammation there is activation of effector cells like macrophages by for example bacterial polysaccharides, and inflammatory cytokines are produced. The outcome is dependent on the quantity of the cytokines in the immediate local environment of the cell or tissue. This is determined by the short half life of the cytokine, and by the neutralising effect of its inhibitor(s). Measurement of circulating cytokines in infection and rheumatic diseases (for example rheumatoid arthritis) have shown correlation with disease activity. For example, the TNF-α concentration is high in endotoxin shock, in the cerebrospinal fluid of cerebral malaria, and in the plasma of active rheumatoid arthritis. In the latter case, both IL-1 and IL-6 also parallel the disease activity.[1] A transgenic mouse model of TNF-α over expressed in the joint has shown classic erosive arthritis that mimics rheumatoid arthritis. However, the case for TNF-α being the 'master cytokine' is not proved in rheumatoid arthritis where all three cytokines are always found at the same time. These results emphasise the limited use of measuring circulating cytokines in rheumatic

Table 1 Monocyte and lymphocyte derived cytokines

Monocyte derived cytokines	*Lymphocyte derived cytokines*
Interleukin-1α (IL-1α)	Interleukin-2 (IL-2)
Interleukin-β (IL-1β)	Interleukin-3 (IL-3)
Interleukin-6 (IL-6)	Interleukin-4 (IL-4)
Interleukin-8 (IL-8)	Interleukin-5 (IL-5)
Interleukin-11 (IL-11)	Interleukin-9 (IL-9)
Tumour necrosis factor-α (TNF-α)	Interleukin-10 (IL-10)
	Interleukin-12 (IL-12)

Table 2 Comparison of IL-1, TNF-α, and IL-6

Biological property	*IL-1*	*TNF-α*	*IL-6*
Endogenous pyrogen fever	+	+	+
Hepatic acute phase proteins	+	+	+
T and B cell activation	+	+	+
Non-specific resistance to infection	+	+	+
Stem cell activation	+	–	+
Fibroblast proliferation	+	+	–
Slow wave sleep	+	+	–
Cyclo-oxygenase; PLA_2 gene expression	+	+	–
Synovial cell activation	+	+	–
Endothelial cell activation	+	+	–
Shock syndome	+	+	–
Induction of IL-1, TNF-α, IL-6, and IL-8	+	+	–
Cell adhesion	+	+	–



Haematopoietic growth factors. EPO=erythropoietin; G-CSF=granulocyte colony stimulating factor; GM-CSF=granulocyte macrophage colony stimulating factor.

disease. Nevertheless, there is still a need to do so, especially in relation to the ratio between cytokines and their inhibitors to determine whether these ratios correlate with activity.[2] If so, redressing the balance would be an obvious therapeutic approach.

Some technical problems with measuring cytokines in biological samples

As mentioned previously, the half life of IL-1β and TNF-α is extremely short. Thus to prevent protein degradation ex vivo, protease inhibitors like EDTA and aprotinin should be added immediately to the freshly drawn blood. The prevention of clotting also will prevent activation of leucocytes during clotting, and subsequent artificial production of cytokines. There is still controversy currently as to whether this in fact is only a theoretical consideration or whether it has real practical consequences and needs to be evaluated by comparing plasma and serum estimations.

A further complication arises if cells from the blood are taken to be cultured in vitro for various experimental analyses. The separation procedure and also the incubation medium should be completely free of endotoxin, otherwise the cells are activated to produce cytokines by endotoxins and therefore results are meaningless.

Cytokine measurements in juvenile chronic arthritis

IL-1 and inhibitor activity was first measured in systemic juvenile chronic arthritis by A-M Prieur and colleagues,[3] where they found IL-1 during febrile episodes, but the sera and urine have IL-1 inhibitory activity. Later, de Benedetti and colleagues have shown that serum IL-6 concentrations correlate with joint involvement and thrombocytosis in systemic juvenile chronic arthritis patients.[4] Our group has reported correlation of IL-1β with joint count in pauciarticular and polyarticular groups in a cross sectional study, but not in the case of systemic juvenile chronic arthritis.[5] When serial measurements of the three cytokines during a peak of fever were made, we were able to see disassociation of the three cytokines with only IL-6 mirroring the fever curve. Subsequently 24 hour profiles on two systemic patients were done, and we have shown that both IL-6 and IL-1ra follow the fever curve. In contrast, the concentrations of IL-1β and TNF-α were barely detectable during this time. These very preliminary results suggest to us that during the fever phase there might be compartmentalisation of the inflammatory cytokines within the central nervous system which would increase the fever. Alternatively, 'the pathogenic cytokine' is IL-6.

Consequences of prolonged unopposed action of inflammatory cytokines

In the chronic rheumatic diseases, it is clear that there are continual expression of these cytokines both in the blood of systemic juvenile chronic arthritis, and in the joints of adult rheumatoid arthritis. Three complications could be construed to be a result of these prolonged cytokine actions: (1) bone destruction, (2) growth retardation, and (3) amyloidosis in susceptible individuals.

(1) BONE DESTRUCTION

IL-1 and TNF-α have been shown to activate collagenase in cartilage cultures. Also in tissue culture IL-1 has been shown to induce porosis and resorption of cartilage and bone. The periarticular osteoporosis seen radiologically around an active swollen joint could be a direct or indirect effect of the cytokines within the joint. As for the generalised osteoporosis in children who have active disease but are not on steroids, again circulating cytokines may be involved; clearly this is an area of which further research needs to be done.

(2) GROWTH RETARDATION

Growth retardation has been well recognised as a serious complication of juvenile arthritis with far reaching social developmental and emotional consequences for a child. Previous work from this unit and others has shown that this can occur in the presence of active disease, steroid treatment compounds the problem, but by no means accounts for all of it.[6 7] Single point measurements of growth hormone has been equivocal between various studies and a more detailed study recently from our unit has shown that 24 hour growth hormone secretion profiles in 20 patients is similar to those profiles of normal children with short stature – that is, they do not appear to be growth hormone deficient.[8] On the other hand insulin growth factor-1 (IGF-1), which mediates growth hormone action

on the target cells, has been shown by many studies to be low in growth retarded children with juvenile chronic arthritis. Our recent study has demonstrated that the concentration of IGF-1 correlates significantly with the clinical measures of disease activity as well as laboratory measures of activity, for example C reactive protein (U Davies, J Jones, J Green, *et al*; unpublished). Furthermore, nutrition can affect IGF-1 concentrations but has been estimated to be normal from 16 diet histories in this study. Therefore it is likely that the process of inflammatory disease has altered the expression of this growth factor, which is responsible for osteoblast activity as measured by osteocalcin. In a recent study where we treated these children with recombinant growth hormone for a year, increase in height velocity correlated with the increase in IGF-1 as well as osteocalcin concentrations (unpublished). Thus IGF-1 is central in the mechanism of growth retardation. Further work is now in progress to unravel the mechanism of low plasma concentrations of IGF-1 in these growth retarded children.

(3) AMYLOIDOSIS

Amyloidosis remains the most common cause of mortality in juvenile chronic arthritis in the last three decades, although there is some clinical evidence that this might be declining with better and earlier management of the disease activity. From two series the systemic juvenile chronic arthritis subgroup seem to be worse affected with this complication,[9 10] and the incidence has been estimated between 5–10%. The fibrous deposit is a polymer of the acute phase protein serum amyloid A. This is an apolipoprotein found mainly in HDL3 fraction of plasma. It is normally undetectable in the serum but can rise to 1000-fold during an acute phase response and chronic inflammation like systemic juvenile chronic arthritis. The regulation of this particular protein, or rather family of proteins, is by the inflammatory cytokines IL-1, TNF-α, and IL-6. Work from this unit has shown that the gene expression of serum amyloid A is mainly transcriptional, and therefore a prolonged cytokine stimulus on hepatocytes will result in persistent high concentrations of serum amyloid A and risk of amyloidosis in susceptible individuals.

Cytokines in other connective tissue diseases

Very little work has been carried out in other connective tissue diseases in childhood. However, systemic lupus erythematosus is the same disease in childhood in terms of clinical manifestations and progression, and laboratory markers. In adult studies polymorphisms of the TNF-α gene seem to be an interesting area in defining individuals that might be susceptible to renal disease. Also studies on the T cell growth factor IL-2 has shown abnormalities in IL-2 expression. As a result the T cell repertoire may not be appropriate and this is currently the subject of research in many laboratories. Furthermore, TGF-β_1 expression is also currently being investigated in relation to the other cytokines it inhibits. The homoeostatic network mechanism and the dysregulation of that homoeostasis is beginning to become a common theme in the various autoimmune diseases.

In adult scleroderma increased expression of the growth factor for fibroblast, platelet derived growth factor, as well as TGF-β_1 have long been noted and has tantalised researchers for many years. Juvenile scleroderma tends to have a slightly different spectrum of disease consisting mainly of localised disease.[11] This is currently the subject of a clinical collaborative study in the UK and whether any of these diseases mimics adult disease remains to be resolved.

Possibilities of cytokine gene therapy?

The concept of replacement and redressing imbalances in the cytokine network is the rationale for cytokine therapy. A simple case of replacement has been very successful in the use of erythropoietin in the treatment of chronic anaemia in renal failure. Erythropoietin has received limited trial in the chronic anaemia of rheumatoid arthritis with some good results and also in juvenile chronic arthritis. In the latter case it appears that erythropoietin works well when there is a concurrent infusion of iron.[12] This obviously poses another problem as to what is the effect of repeated iron load on the bone marrow as well as whether it will cause flare-ups of arthritis, as iron is an oxidant.

In the field of redressing imbalance in the cytokine network, antibodies to TNF-α have been successfully used in counteracting septicaemic shock and recently a short term trial has been done on rheumatoid arthritis with good clinical results (M Feldmann, R Maini, personal communication). The problem is relapses and subsequent antibody formed against the chimeric TNF-α antibody. IL-1ra also has been used with good effect in septic shock and also produced transient relief in rheumatoid arthritis when given in large quantities. The problem with using receptor blockade is that cytokines can probably work through only a few receptors and therefore one would need to blockade the majority of receptors to produce the desired effect. Recycling of receptors will obviously shorten the effect of the receptor antagonist as well. TGF-β_1 has been used successfully in animal studies to promote skin growth but there are no trials as yet on autoimmune diseases.

In conclusion, therefore, until the cytokine network is more clearly understood, cytokine therapy is still experimental. The fact that in the majority of rheumatic diseases all three cytokines are over expressed suggests that blockade of a common cellular pathway would be a much more ideal method than blockade of individual cytokines. Further research is needed to see if this might be possible.

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