It is now widely accepted that the investigation of cytokine expression during inflammation can provide information about the pathogenesis of disease and help to define key therapeutic targets [1]. These studies have been greatly facilitated by the availability of recombinant human cytokines, essential for the development of specific assays. As assays have become more widely available, rheumatologists have applied them to study arthritis, taking advantage of access to biological fluids such as synovial fluid at the site of inflammation, as well as those distant from the inflamed tissue.

To date there is little doubt that in synovial fluid (SF), pro-inflammatory cytokines such as interleukin-1 (IL-1) [2], interleukin-6 (IL-6) [3], interleukin-8 (IL-8) [4], granulocyte macrophage colony stimulating factor (GM-CSF) [5] and tumour necrosis factor alpha (TNFα) [6] are detectable in abundance, whilst the T cell cytokines IL-2, IL-4, interferon-γ and lymphotoxin are virtually undetectable [7]. However, an overview of the many studies cataloguing cytokine levels in biological fluids has revealed that there are differences regarding the relative levels of some of these cytokines. Furthermore, the presence of a cytokine at high levels, such as IL-6, does not necessarily indicate a pathogenic role. Over the past few years it has become clear that interpretation of these studies is dependant upon the assays used, and an awareness of the many factors which interfere within each specific system.

Almost without exception, cytokines have been characterized on the basis of their biological actions. Thus, bioassays preceded the development of immunoassays, many studies focusing on detection of cytokines in tissue culture supernatants. However, the pleiotropic nature of these mediators is reflected in the observation that bioassays are not cytokine specific. For example, the mouse thymocyte proliferation assay used for measurement of human IL-1, also respond to human IL-2, IL-6, and IL-7 [8-10] and even more murine cytokines e.g. IL-4, TNF and LT. Furthermore when bioassays are used to measure cytokines in biological fluids, other problems are encountered. For example, the effects of body fluids on promoting (or inhibiting) cell growth has to be considered, and specificity has to be confirmed with neutralizing antibodies. As well as being labour intensive, bioassays lack reproducibility, an important criterion if assays are to be used for clinical evaluation. Furthermore, an awareness of the presence of specific inhibitors such as soluble cytokine inhibitors which interfere with cytokine bioactivity, is of importance (reviewed in [11]).

The development of high affinity monoclonal antibodies (mAb) to cytokines for use in immunoassays meant that they could be performed rapidly, with a high degree of reproducibility and specificity, although it is clear that the detection limit of these assays is often not as low as the level at which cytokines function biologically. Furthermore, detailed analysis of these techniques demonstrated clearly that inhibitory factors were not confined to bioassays. Thus, non-specific serum proteins such as α2-macroglobulin can bind to IL-1, IL-2, IL-6, platelet derived growth factor and fibroblast growth factor β [12] mask epitopes, and therefore block detection in immunoassays. For this reason chloroform extraction, or silica adsorption have been recommended prior to assay of IL-1β in plasma [13]. Rheumatoid factors (RF) are also well recognized as a cause of false positive results in immunoassays, although techniques have been applied for extracting RF such as reduction and alkylation of samples prior to assaying [14].

Despite overcoming these physiochemical hurdles, most immunoassays are limited in that they give little information regarding levels of biologically active protein. Holt et al. in this issue compares levels of bioactive IL-1 with immunoreactive levels in SF and plasma of arthritic patients, and finds large discrepancies. These observations have also been described for other cytokines such as TNFα [15]. Such discrepancies have been shown to be due, at least in part, to the presence of specific inhibitors which themselves are critical components of cytokine regulatory networks. Thus, the balance between cytokines and levels of their respective inhibitors will determine bioactivity [11]. In the case of IL-1 these inhibitors include both the IL-1 receptor antagonist (IL-1ra) [16], but also shed IL-1 receptor [17]. The important regulatory role of these cytokine inhibitors in vivo is further substantiated by the observation that following infusion of TNFα into cancer patients, a transient rise and fall of serum TNFα is observed within 4-6 h, which is accompanied by a rapid rise in soluble TNF receptor levels, still detectable after several days [18]. Similar observations have been described for IL-1β and IL-1ra in healthy volunteers injected with endotoxin [19]. These studies indicate that levels of cytokine inhibitors possibly acting homeostatically reflect enhanced expression of cytokines in vivo, and their measurement could be of more value than that of the cytokines themselves.

Soluble receptors have now been described for many cytokines including IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, TNF, CSF-1, and interferon-γ (reviewed in [11]), and are known to represent truncated forms of membrane receptors lacking transmembrane and intracytoplasmic domains. The best characterized binding protein is the soluble IL-2R (sIL-2R), which represents the shed low affinity IL-2R α chain (Tac antigen) [20]. Although reports have described associations between sIL-2R levels and the severity or prognosis of autoimmune [21]
and malignant disease [22], the physiological significance of such low affinity inhibitors remains uncertain.

Our laboratory has recently reported elevated levels of TNF binding proteins (p55 and p75 soluble TNF-Rs [sTNF-Rs]) in the serum and synovial fluid of patients with rheumatic disease using two specific ELISAs developed by Wallach and colleagues [23]. In contrast to sIL-2Rs, these TNF inhibitors bind TNFα with high affinity. Both sTNF-Rs are produced locally in synovial joints, are highest in synovial fluid from RA patients, and are able to inhibit TNF cytotoxicity in vitro. Furthermore, unlike TNFα, sTNF-R levels correlated closely with disease activity in both serum and SF. Interestingly, unlike most cytokines, nanogram quantities of sTNF-Rs are detectable in serum of healthy individuals, levels of which vary surprisingly little over prolonged periods of time. At present the precise functional significance of cytokine inhibitors in vivo is unclear, although recent studies (W. Buurman, personal communication) have shown that sTNF-Rs may play an important role in the clearance of TNFα from the circulation. Thus, immunoreactive TNFα persisted in the serum of bilaterally nephrectomized mice, although this material was biologically inactive. Indeed, serum sTNF-Rs were found to be raised in these mice compared to sham-operated animals, in whom TNFα levels were expressed only transiently.

Pharmacokinetic and pharmacodynamic studies of cytokine/inhibitor interactions will be possible when the relevant murine immunoassays for these molecules become available. It may then be possible to determine whether over-expression of cytokines in chronic inflammatory states, is related to inherent abnormalities in the production of the relevant inhibitors. In the meantime one must be cautious about measuring short range mediators from biological fluids distant from the site of inflammation and be aware of the many limitations specific to each assay system.

A. P. COPE and F. M. BRENNAN
The Kennedy Institute of Rheumatology, incorporating the Charing Cross Sunley Research Centre, Lurgan Avenue, Hammersmith, London W6 8LW