

Showcase on Research

Macrophages as Critical Targets in Chronic Inflammation

John A. Hamilton¹ and Gary Anderson²¹Arthritis and Inflammation Research Centre, The University of Melbourne Department of Medicine, Royal Melbourne Hospital, Parkville, VIC 3050²Departments of Medicine (RMH) and Pharmacology, University of Melbourne, Parkville, VIC 3010**Macrophages and chronic inflammation**

Macrophage lineage cells are key components of inflammatory reactions particularly those of a chronic nature. For example, in the inflamed joints of patient with rheumatoid arthritis, their numbers correlate with the degree of joint damage and they are the producers of important proinflammatory mediators, such as TNF α and IL-1. The reasons why certain inflammatory lesions proceed to a chronic state, thereby leading to pathology, are unknown.

Macrophage-colony stimulating factor (M-CSF) and granulocyte macrophage-CSF (GM-CSF)

Both M-CSF (CSF-1) and GM-CSF were originally defined by their ability to form myeloid colonies from precursor cells in the bone marrow (1). However, they can also affect the functions of more mature cells, e.g. macrophages and/or granulocytes. We suggested some time ago that they should also be regarded as proinflammatory cytokines because of their ability to stimulate urokinase-type plasminogen activator (u-PA) activity in macrophages (2).

"CSF network" hypothesis

As one possibility to explain chronicity, as well as the increased macrophage numbers in inflammatory lesions (e.g. the rheumatoid synovium), we have proposed a "CSF network" hypothesis in which CSF-1 and GM-CSF are key inflammatory cytokines and therefore worthy of targeting (Fig. 1) (3,4). This positive feedback loop with these CSFs controlling local macrophage survival, proliferation and activation was based in part on *in vitro* findings showing that (i) the CSFs can have these functions, (ii) macrophage-derived cytokines, such as TNF α and IL-1, can stimulate CSF formation from many cell types and (iii) the CSFs are widely present at sites of

inflammation. A consequence of this hypothesis is that more macrophages would be generated at a site of inflammation to produce more inflammatory mediators - in other words, a cycle can be established, perhaps contributing to the chronicity. If the hypothesis has validity, then it would mean that targeting CSFs would result in disruption of the network with fewer and/or less active macrophages available to produce inflammatory cytokines and other mediators, such as u-PA. Supporting evidence has subsequently been obtained for this hypothesis, using gene-deficient mice and blocking antibody strategies, some of which is summarised below (5-12).

Arthritis models

We have used two murine arthritis models, the collagen-induced model and the so-called methylated BSA/IL-1 model. The former is a chronic and systemic model while the latter is an acute, monoarticular one. In both models the CSFs exacerbated disease (5,7,8) while removal of CSF action, using gene knockout and antibody blockade strategies, prevented and/or alleviated

disease progression (6,8-10,12). Data for the preventative action of GM-CSF blockade with a monoclonal antibody are given in Fig. 2. The degree of inhibition of the arthritis in the systemic model following anti-GM-CSF therapy was more profound than the corresponding data obtained after TNF α blockade (13) - it is worth noting that anti-TNF α therapy is proving to be reasonably successful in the treatment of rheumatoid arthritis.

One other prediction from the hypothesis is that there would be a mutual interdependence between CSF levels and those of IL-1 and TNF α . Studies with GM-CSF targeting have confirmed this concept (9,10,12). It is also predicted that CSF blockade would be effective because there would be less cells available to produce TNF α , IL-1 and so on, thus obviating the need for combination therapies targeting both of those other mediators.

Again using gene-knockout studies, we have implicated u-PA in the collagen-induced arthritis model (10), an observation we are proposing to be consistent with the "CSF network" hypothesis (Fig. 1).

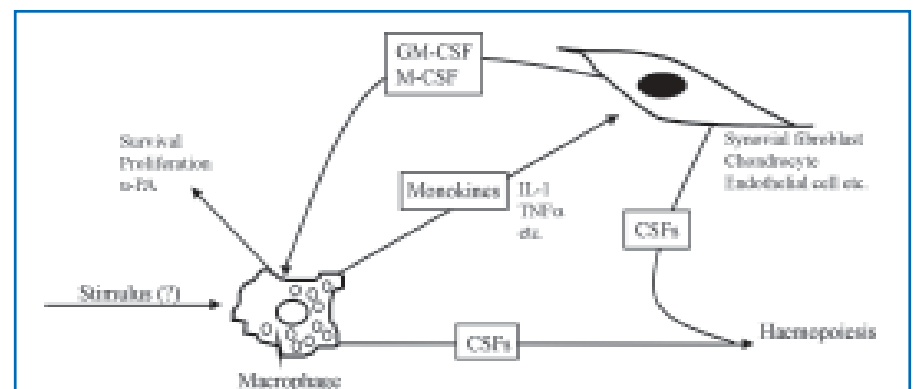


Fig. 1. A proposed proinflammatory CSF-dependent cytokine network. During an inflammatory reaction in tissues (such as joints), appropriately stimulated macrophages can activate neighbouring non-hemopoietic cells, for example via IL-1 and TNF α , to synthesise GM-CSF and M-CSF. The secreted myeloid cell regulators can, in turn, increase the numbers of activated macrophages, following enhanced survival and even local proliferation, capable of releasing further inflammatory mediators, such as u-PA, and thereby resulting in a chronic cascade. Enhanced haemopoiesis may also ensue leading to increased numbers of macrophages and PMNs. Predictions from this network model of chronicity are as follows. First, CSF depletion would have an anti-inflammatory benefit by reducing the numbers of activated macrophages and PMNs capable of participating at a site of inflammation. Second, there is a mutual dependence of CSF levels, on the one hand, and of those of IL-1 and TNF α , on the other. This diagram is an adaptation of one published previously (3,4).

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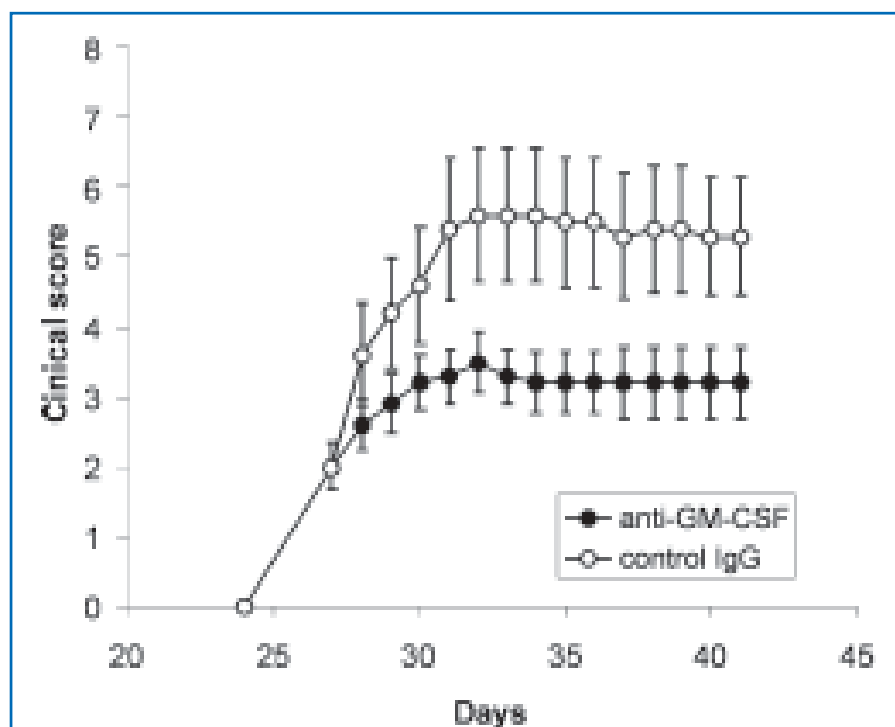


Fig. 2. Anti-GM-CSF antibodies block CIA development. DBA/1 mice were boosted with collagen type II on day 21 and treated on day 27 with 300 mg (i.p. daily for 10 days) monoclonal anti-GM-CSF or IgG isotype control. Daily clinical scores (mean (SEM), n = 10/group) were significantly lower; χ^2 test.

Experimental allergic encephalomyelitis (EAE)

In collaboration with the laboratory of Prof. C. Bernard, La Trobe University, we have found a dramatic reduction in EAE disease (a multiple sclerosis model) using GM-CSF^{-/-} mice or normal mice again treated with monoclonal antibody to GM-CSF (11).

Lung inflammation

Chronic inflammatory diseases of the lung are amongst the most prevalent and costly health disorders in the global health-care system. Research in our laboratories is directed towards trying to understand the molecular basis of two of these diseases, namely asthma and chronic obstructive pulmonary disease (COPD). One focus of our research is to identify and then to modify candidate genes that might be determinants of either the severity or the persistence of the inflammation. To achieve this, our laboratory has specialised in the development of mouse models of chronic inflammatory lung diseases to model specific traits that occur in human lung disease. Recent evidence strongly suggests that macrophages are likely to play a key role in the molecular pathogenesis of these disorders.

COPD will be a third major cause of death globally by the year 2010. The term COPD actually refers to a group of conditions: emphysema (proteolytic lung destruction), bronchiolitis (small airway fibrosis) and bronchitis (mucus hypersecretion) (14,15). In contrast to asthma, where the primary cause(s) is obscure, it has been recognised for decades that the main cause of COPD in the community is cigarette smoking. However, the essential conundrum is that, while smokers develop airway inflammation, only about 10 per cent develop COPD. In a new COPD exacerbation (16) model that we have developed, using intranasal administration of lipopolysaccharide, it was observed that anti-GM-CSF monoclonal antibody blocked dramatically the macrophage accumulation and proliferation, as well as the neutrophilia, even when administered (therapeutically) after the insult (Anderson, G.P., Cook, A.D., and Hamilton, J.A., unpublished work). After antibody treatment, leading to reduced GM-CSF levels (ELISA and bioactivity), the number of proliferating macrophages (mitotic figures) was reduced, and TNF α levels in the alveolar fluid were lowered (ELISA); enhanced PMN apoptosis (17,18) was noted and importantly

matrix metalloproteinase levels were markedly suppressed. This therapeutic concept has not previously been considered for COPD (15).

Asthma is an inflammatory disease of the airways affecting predominantly the small bronchi that are the sites of resistance to airflow in the lungs. Asthma causes the small airways to become inflamed and swollen, and airway smooth muscle to become excessively sensitive to diverse and constricting stimuli. It has been recognised for over a century that allergy is frequently associated with asthma. Moreover, it has been recognised for a longer time that the specific trait, eosinophilia, is very frequently associated with asthma. For decades the molecular basis of these traits was obscure. It is now well understood that the allergic inflammatory component of asthma, i.e. the characteristic eosinophilic inflammation of the airway mucosa with mast cell hyperplasia, can be largely explained by immune deviation favouring TH2-biased immunity where net IL-4, IL-13, IL-5 and IL-9 cytokine induction predominates (19). However, the TH2 model (20) does not adequately explain the observation that asthmatics often undergo severe and sudden worsening (exacerbations) of their condition associated with airway and tissue neutrophilia, and IFN γ induction (21,22), which may be triggered in humans by airway infection with viruses or other pathogen (23). It is therefore of considerable interest that we have demonstrated recently that neutralising anti-GM-CSF antibodies are highly effective in models of pure TH2-driven allergic asthma and also newly developed models of disease exacerbation. One reason for the high therapeutic efficacy of anti-GM-CSF antibody in these models may be that GM-CSF is released in high amounts from both TH1- and TH2-biased lymphocyte subsets as well as epithelial and stromal tissue. Given the significance of GM-CSF for regulating macrophage biology, it is highly likely that macrophages are relevant across the entire clinical spectrum of asthma and not only to the most severe forms, which until recently has been assumed to be the case (24).

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Macrophages as Critical Targets in Chronic Inflammation (contin.)

Effect of "persistent" particulates, including adjuvants

As an additional mechanism to account for chronic stimulation of macrophage-lineage cells, we have also made the following proposals. Certain poorly degradable particulates (e.g. oxidised LDL, talc, amyloid fibrils, calcium phosphate crystals, adjuvants (alum, oil in water emulsions)) promote macrophage survival (and low levels of DNA synthesis), thereby increasing macrophage numbers in lesions. This would help to explain the chronicity of certain inflammatory reactions (e.g. atherosclerotic plaques, granulomas, synovitis, Alzheimer's disease) (25-31) (Fig. 3). A possible mechanism for the mode of action of particulate adjuvants was also offered and for the adjuvant action of GM-CSF (26). We also found that these particulates, in the presence of low (suboptimal) CSF-1 concentrations, potentiated dramatically DNA synthesis represented by the macrophages, having taken up the particulate, proliferating in response to CSF-1 doses which normally promoted mainly survival (Fig. 3). Studies using macrophages from op/op (CSF-1-deficient)/GM-CSF^{-/-} mice indicated that neither endogenous CSF-1 nor GM-CSF was responsible.

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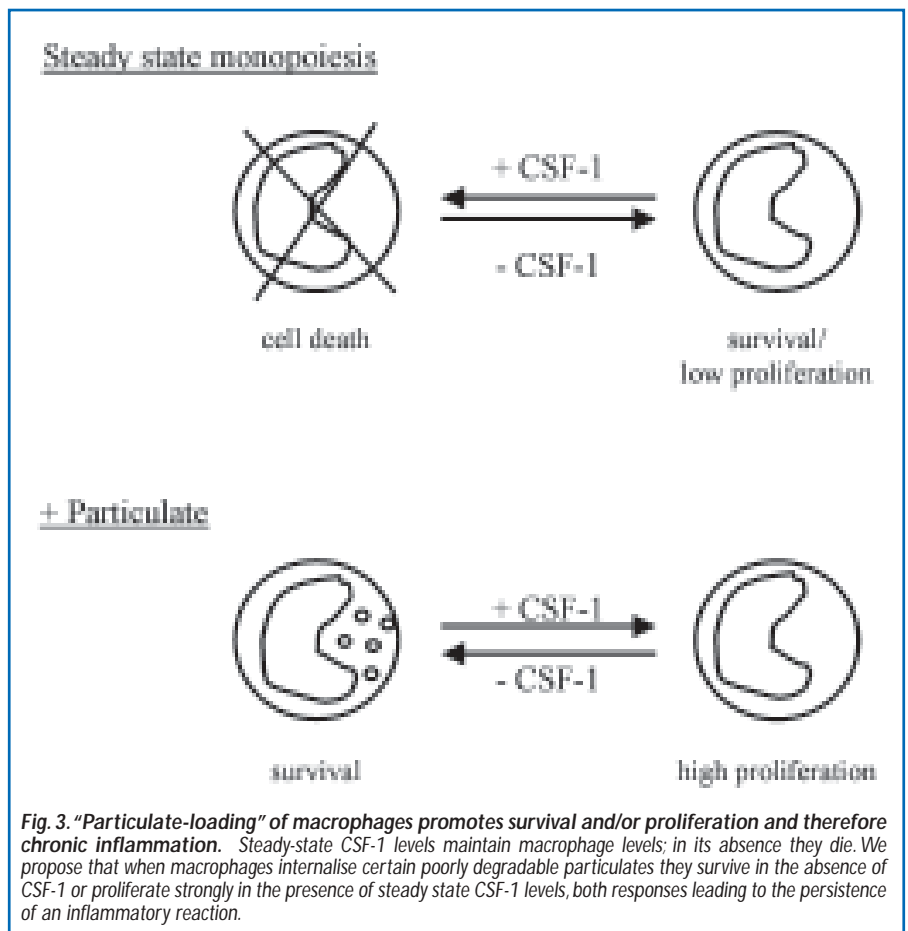


Fig. 3. "Particulate-loading" of macrophages promotes survival and/or proliferation and therefore chronic inflammation. Steady-state CSF-1 levels maintain macrophage levels; in its absence they die. We propose that when macrophages internalise certain poorly degradable particulates they survive in the absence of CSF-1 or proliferate strongly in the presence of steady state CSF-1 levels, both responses leading to the persistence of an inflammatory reaction.

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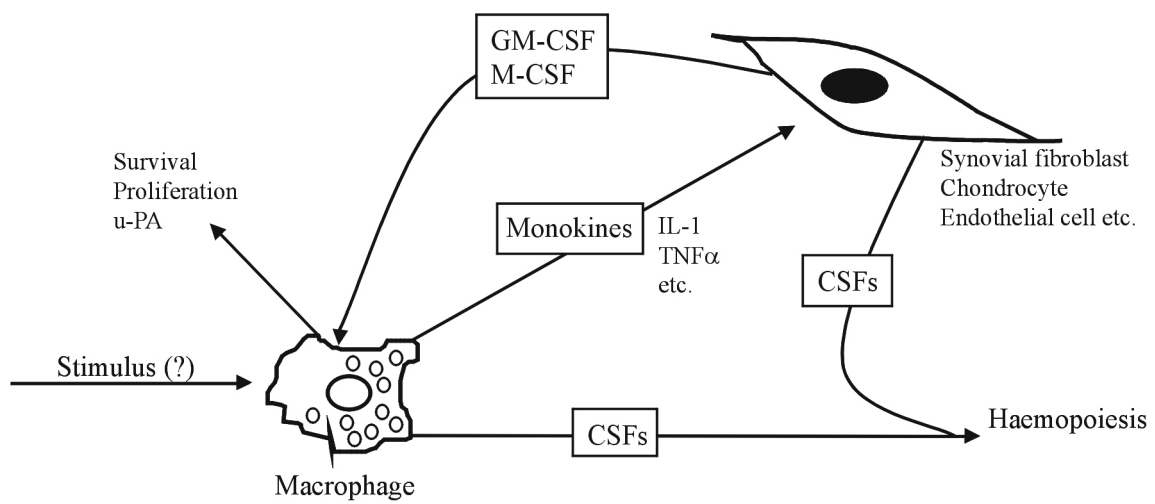


Fig. 1

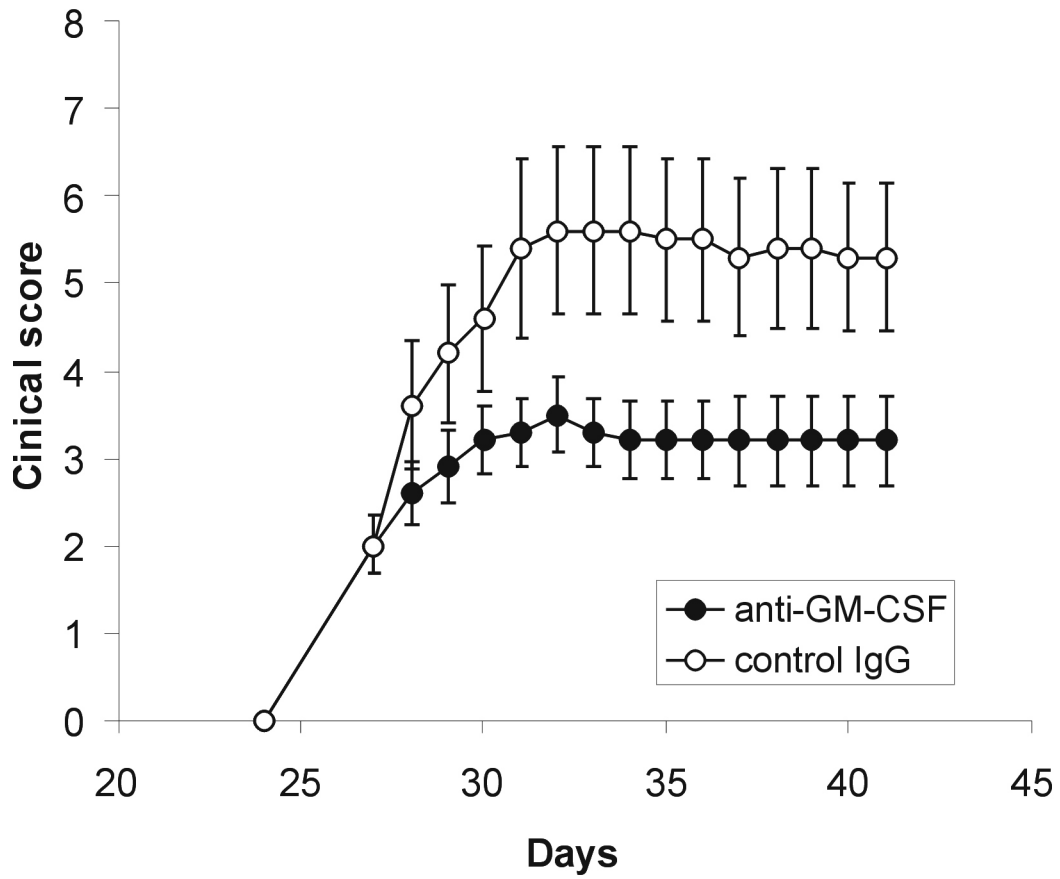


Fig. 2

