

## **EDITORIAL**

# **Eosinophil priming and migration in idiopathic pulmonary fibrosis**

C. Kroegel, M. Foerster, P.R. Grahmann, R. Braun

Although neutrophils are considered to be the major effector cells in idiopathic pulmonary fibrosis (IPF) [1], accumulation of eosinophils in both alveolar spaces and the parenchyma is characteristically also detected [2]. In addition, raised eosinophil numbers are usually found in the bronchoalveolar lavage (BAL) fluid obtained from patients with IPF, and in approximately one third of the patients, exceed those of neutrophils [3]. Further, eosinophil numbers closely correlate with the severity of clinical impairment as assessed by symptom score, radiological and lung function abnormalities [4, 5]. Finally, raised concentrations of the granular eosinophil cationic protein (ECP) were detected in patients when compared to healthy controls [5, 6]. These observations suggest that eosinophils represent an integral part of the inflammatory cell reaction in IPF. They also suggest that in IPF, eosinophils become activated or primed either during infiltration and/or residence in the airways. Therefore, one would expect to find eosinophil-activating or priming cytokines being expressed and secreted within the airways of patients suffering from IPF. Unfortunately, to date we do not know the factors linking eosinophil recruitment and activation to the pathogenetic processes in IPF.

What might those factors be? There are a number of potentially specific and nonspecific chemoattractants that may be involved in leukocyte recruitment into the lung of patients with IPF. These include interleukin (IL)-1, IL-8, IL-15, granulocyte-macrophage colony-stimulating factor (GM-CSF), the protein that is regulated on activation with normal T-cell expression and secretion (RANTES), leukotriene B<sub>4</sub> (LTB<sub>4</sub>), macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ ), and monocyte chemoattractant protein-1 (MCP-1) (for review see [7]). In addition, the superfamily of tumour necrosis factor (TNF)-like ligands represent another emerging family of cytokines that appears to be important in the regulation of eosinophil accumulation in the lung [8]. As yet, however, only few attempts have been undertaken to identify the factors operative in IPF.

In order to provide further insight into the potential operating mechanisms, a recent study by BOOMARS *et al.* [9] published in this issue of the Journal assessed the migratory response of eosinophils to BAL fluid from patients with IPF in whom significantly increased numbers of BAL eosinophils were detected. They found that peripheral blood eosinophils obtained from healthy volunteers did not migrate when exposed to both unconcentrated and concentrated BAL fluid recovered from IPF patients. However, when blood eosinophils were preincubated with low

concentrations of GM-CSF ( $10^{-11}$  M) for 30 min at 37°C, a chemotactic response towards the BAL fluid was elicited. When anti-IL-8 and anti-RANTES antibodies were added, chemotactic activity to BAL fluid from patients suffering from IPF remained unchanged, indicating that the two cytokines are not responsible for the effect observed. Finally, BAL fluid did not alter basal intracellular calcium concentration, irrespective of whether cells were primed or not, which further reduces the number of potential candidates to those acting without a significant alteration of the intracellular calcium level.

So far so good. Surprisingly, however, a comparable chemotactic response could also be induced when primed eosinophils were exposed to BAL fluid obtained from healthy controls without lung disease. Moreover, as with BAL fluid recovered from patients with IPF, primed blood eosinophils failed to show any change in the intracellular calcium level, and the magnitude of the chemotactic migration was not affected in the presence of anti-IL-8 and anti-RANTES-antibodies. These data suggest that the migratory response to BAL fluid was not due to a specific immune mechanism operative in IPF, but merely reflected the altered functional properties of eosinophils caused by the effect of submaximal concentration of GM-CSF on the cells during *in vivo* incubation.

The paper by BOOMARS *et al.* [9], allows a number of conclusions and raises several interesting issues which may be relevant for designing future investigative studies employing BAL fluid. Firstly, in the face of it, the data suggest that BAL fluid even from healthy subjects contains a chemotactic factor for eosinophils. As pointed out by the authors, this biological activity may be involved in the physiological immune surveillance within the pulmonary compartment. The production of low amounts of pro-inflammatory mediators under physiological conditions is an interesting aspect which we tend to forget.

Secondly, a possible reason for the comparable chemotactic response induced by BAL fluid from patients with IPF and healthy subjects may be due to the method chosen for assessing the functional response of eosinophils. It has been recognized that cell recruitment from the circulation into the tissue is a complex process which, beside activation of chemotactic locomotion, essentially requires the induction of adhesive mechanisms by endothelial cells. When filters are applied to separate the lower and upper compartment of the Boyden chamber, the endothelial-dependent part of the chemotaxis is not accounted for. Therefore, on the basis of the experimental design of the study, it is possible that additional factors only contained in the BAL fluid from patients with IPF may have facilitated a chemotactic response *via* activation of endothelial-dependent mechanisms.

Thirdly, the data presented by BOOMARS *et al.* [9] suggest that among the factors potentially responsible for the accumulation and activation of eosinophils, both IL-8 and RANTES can be excluded. Since the chemotactic response elicited by BAL fluid was not accompanied by an alteration in intracellular calcium levels, factors inducing intracellular calcium mobilization, such as LTB<sub>4</sub>, platelet activating factor (PAF), C5a and the pro-inflammatory members of the chemokine family, can also be removed from the list of possible mediators [7]. Put another way, according to the results presented by BOOMARS *et al.* [9], cytokines such as IL-1, IL-15 or TNF- $\alpha$  appear to be the more likely candidates operative in IPF.

Data from another recent study [8] investigating the role of TNF- $\alpha$  in an experimental mouse model of pulmonary fibrosis appear to support this conclusion. The study demonstrates that a monoclonal anti-TNF- $\alpha$  antibody not only suppresses the extent of pulmonary fibrosis and tissue eosinophilia but also the expression of transforming growth factor- $\beta$  (TGF- $\beta$ ) and IL-5 mRNA. Since IL-5 is known to potently mediate differentiation, activation and recruitment of eosinophils, the data indicate a novel mechanism by which TNF- $\alpha$  could facilitate eosinophil recruitment *in vivo*.

This observation corresponds to other findings showing that TNF- $\alpha$  prolongs eosinophil survival *in vitro* [10], potentiates eosinophil synthesis of leukotriene C<sub>4</sub> [11], increases eosinophil cytotoxicity toward *Schistosoma mansoni* larvae [12], and enhances eosinophil adhesion to endothelial cells [13]. Even more interestingly, the activity of TNF- $\alpha$  on eosinophils has been demonstrated to synergize with that of IL-5 [14]. In view of the effect of TNF- $\alpha$  on IL-5 expression [8], this interaction may be of particular pathogenetic relevance and may explain, at least in part, the eosinophil recruitment observed in IPF.

Fourthly, the latter finding points to another possible explanation for the failure to detect a possible difference between BAL fluid from normals and IPF patients. The experimental design does not take into account the potential effect of the cytokine networking, where one cytokine may transmit the information to a second cell which, in turn, secretes the relevant messenger for the target cell.

Fifthly, a different reason may relate to the handling of BAL fluid. Although studies employing BAL fluid have provided extremely useful information on the pathogenetic mechanisms operative in obstructive and restrictive lung disease, the technique is not as straightforward as it might appear. For instance, BAL fluid handling procedures may affect the cellular, protein and mediator content of the BAL aspirate, which include the time elapsed before freezing or processing of the samples. In addition to the technical considerations, BAL fluid constituents may be altered spontaneously following removal from the lung compartment, *i.e.* spontaneous degradation of unstable lipid mediators or proteolysis of protein compounds. As a result, the original immunological quality of the fluid may change after removal, even if rapid handling and other precautionary measures are applied.

As BAL fluid from normals and patients with IPF are indistinguishable on the basis of inducing a migratory response, the paper also emphasizes the importance of priming for the understanding of inflammatory cell function. For instance, the data presented by BOOMARS *et al.* [9] allows the conclusion that BAL fluid constituents in IPF are not

different from those obtained from normal subjects. The sole difference being that in contrast to healthy subjects, patients with IPF secrete more GM-CSF at concentrations that are sufficient to pre-activate or prime eosinophils in the blood, thus allowing the eosinophils to respond to chemoattractants originating within the lung compartment. This scenario appears far fetched, considering that IPF is primarily a fibrosing disease of the lung which essentially requires immunopathological processes to be operative in the lung tissue itself. However, on the basis of the present data, this view cannot be discarded to-tally.

The possibility that eosinophil functions can be modulated in disease has attracted much attention over the past 10 years. Generally, priming describes an increase in the functional response of a cell to a primary stimulus caused by a secondary agonist that, in itself, induces little or no overt cell reaction [15, 16]. Priming of eosinophils may be mediated by soluble agents and adhesion molecules [15, 17]. Furthermore, it is not restricted to a single cell function but covers most, if not all, responses a given cell is capable of such as the respiratory burst, toxicity to host tissue and invading parasites, degranulation, production of lipid mediators, adhesion, and, as shown by BOOMARS *et al.* [9] in this issue of the Journal, chemotaxis. Finally, priming has been demonstrated with several cell types, including basophils, macrophages and neutrophils [18, 19, 20]. Thus, priming can be best viewed as an inherent property of inflammatory cells for an optimal performance within an immunological setting.

Taken together, priming is an extremely important mechanism that explains the apparent discrepancy between the high agonist concentration required to elicit a cell response *in vitro* and the relatively low concentrations achieved *in vivo*. In addition, as indicated by the observations presented by BOOMARS *et al.* [9], priming within the blood compartment may also help to explain why some patients with significant peripheral hypereosinophilia do not develop organ manifestation or symptoms. Nevertheless, it is too easy to conclude that migration of eosinophils is always governed by priming as both the activation state of eosinophils and the role of endothelium was not assessed by the study. However, the approach used by BOOMARS *et al.* [9] should ignite further studies which not only include investigating the BAL fluid from patients with IPF but also the eosinophil itself.

## References

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